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Metabolic and hormonal responses in the regulation of blood glucose levels in infants delivered by elective caesarean section

Koh, Daisy Ko Ming

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Daisy Ko Ming Koh

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**METABOLIC AND HORMONAL RESPONSES IN
THE REGULATION OF BLOOD GLUCOSE
LEVELS IN INFANTS DELIVERED BY
ELECTIVE CAESAREAN SECTION**

Dr. Daisy Ko Ming Koh

Doctor of Medicine

University of Dundee

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TABLE OF CONTENTS

TABLE OF CONTENT	I
LIST OF ILLUSTRATIONS	VI
LIST OF TABLES	VII
ABBREVIATIONS	VIII
ACKNOWLEDGEMENTS	XI
DECLARATION	XII
ABSTRACT	XIII
CHAPTER 1: INTRODUCTION	1
1.1 Cerebral damage and hypoglycaemia	1
1.1.1 Neuropathological consequences of hypoglycaemia	3
1.2 Definition of hypoglycaemia	4
1.2.1 Clinical definition of hypoglycaemia	4
1.2.2 Statistical definition	5
1.2.3 Physiological definition	6
1.2.3.1 Hormonal responses to hypoglycaemia	6
1.2.3.2 Neurophysiological definition	8
1.2.4 Neurodevelopmental approach	9
1.2.5 Conclusion	10
1.3 Fetal metabolism during delivery	11
1.3.1 Perinatal glucose homeostasis	12
1.3.2 Hepatic glycogen stores	13
1.3.3 Hepatic microsomal glucose-6-phosphatase system	13
1.3.3.1 The ontogeny of the human hepatic microsomal glucose-6-phosphatase	15

1.3.3.2	Perinatal factors and the regulation of glucose-6-phosphatase gene expression	16
1.3.4	Perinatal hormonal and metabolic responses to extrauterine life	18
1.3.4.1	The physiological role of amino acids in the perinatal period	19
1.3.4.2	The physiological role of insulin and glucagon in the perinatal period	20
1.3.4.3	The physiological role of cortisol in the perinatal period	22
1.3.4.4	The physiological role and metabolism of catecholamines in the perinatal period	25
1.4	Aim of thesis	34
1.4.1	Objectives	35
CHAPTER 2:	PILOT STUDY 1: GLUCOSE AND LACTATE LEVELS IN CORD BLOOD SAMPLES ROUTINELY COLLECTED AT DELIVERY FOR ESTIMATION OF BLOOD GAS AND ACID-BASE STATUS IN TERM INFANTS	36
2.1	Introduction	36
2.2	Methods	37
2.2.1	Mothers and infants	37
2.2.2	Blood sample collection	38
2.2.3	Data collection	38
2.2.4	Data handling and statistical analyses	39
2.2.5	Biochemical Analysis	40
2.2.5.1	Glucose (mmol/l)	40
2.2.5.2	Lactate (mmol/l)	41
2.2.5.3	Blood gas and acid-base profiles	42
2.3	Results	43

2.4	Commentary	50
CHAPTER 3:	PILOT STUDY 2: MEASURING CHANGES IN	52
	VENOUS CORD BLOOD GLUCOSE, LACTATE,	
	PH, PO₂, PCO₂, BICARBONATE AND BASE	
	EXCESS VALUES WITH TIME FOLLOWING	
	DELIVERY	
3.1	Introduction	52
3.2	Methods	52
3.2.1	Mothers and infants	52
3.2.2	Blood sample collection	53
3.2.3	Statistical analyses	54
3.2.4	Biochemical Analysis	54
3.2.4.1	Glucose (mmol/l)	54
3.2.4.2	Lactate (mmol/l)	55
3.2.4.3	Blood gas and acid-base profiles	55
3.3	Results	55
3.4	Commentary	58
CHAPTER 4:	PILOT STUDY 3: TIME INTERVAL FROM	65
	DELIVERY TO UMBILICAL CORD BLOOD GAS	
	AND ACID-BASE STATUS MEASUREMENT AT	
	DELIVERY: ROUTINE CLINICAL PRACTICE	
4.1	Introduction	65
4.2	Methods	65
4.2.1	Mothers and infants	65
4.2.2	Statistical analyses	66
4.3	Results	66
4.4	Commentary	67
CHAPTER 5:	STUDY 4: METABOLIC AND HORMONAL	71
	RESPONSES IN THE REGULATION OF BLOOD	
	GLUCOSE LEVELS IN INFANTS DELIVERED BY	

ELECTIVE CAESAREAN SECTION

5.1	Introduction	71
5.2	Methods	72
5.2.1	Mothers and infants	72
5.2.2	Blood sample collection	72
5.2.3	Data collection	74
5.2.4	Statistical analyses	75
5.2.5	Biochemical analysis	76
5.2.5.1	Glucose (mmol/l)	76
5.2.5.2	Lactate (mmol/l)	76
5.2.5.3	3-Hydroxybutyrate (mmol/l)	76
5.2.5.4	Free fatty acids (mmol/l)	77
5.2.5.5	Amino acids (μ mol/l)	78
5.2.5.6	Insulin (mU/l)	79
5.2.5.7	Glucagon (ng/l)	80
5.2.5.8	Human growth hormone (mU/l)	81
5.2.5.9	Cortisol (nmol/l)	82
5.2.5.10	Catechols (pg/ml)	83
5.2.5.11	Blood gas and acid-base profiles	85
5.3	Results	85
5.4	Commentary	94
5.4.1	Maternal and fetal glucose homeostasis	94
5.4.2	Lactate and glucose homeostasis in infants	97
5.4.3	Amino acids and glucose homeostasis in infants	103
5.4.4	Glucagon, insulin and glucose homeostasis in infants	104

5.4.5	Cortisol and glucose homeostasis in infants	107
5.4.6	Catechols and glucose homeostasis in infants	109
CHAPTER 6: DISCUSSION		113
REFERENCES		119
APPENDICES		140

LIST OF ILLUSTRATIONS

Figure 1.1	Pathways of metabolism of catecholamines	28
Figure 1.2	Pathways of oxidative deamination of catecholamines	32
Figure 3.1	Venous cord blood glucose, lactate, pH, pO ₂ , pCO ₂ , cHCO ₃ and BE values with time following delivery of infants by elective caesarean section	57

LIST OF TABLES

Table 2.1	Association of maternal antenatal factors with hypoglycaemia (glucose levels less than 2.6 mmol/l) in venous cord blood samples	46
Table 2.2	Association of maternal intrapartum factors with hypoglycaemia (glucose levels less than 2.6 mmol/l) in venous cord blood samples	47
Table 2.3	Association of infant factors with hypoglycaemia (glucose levels less than 2.6 mmol/l) in venous cord blood samples	48
Table 2.4	The relationship of mode of delivery and levels of blood glucose, lactate, pH, pO ₂ , pCO ₂ , bicarbonate and base excess	49
Table 3.1	Venous cord blood glucose, lactate, pH, pO ₂ , pCO ₂ , bicarbonate and base excess values with time following delivery by elective caesarean section	56
Table 4.1	Time taken to measure blood gases after delivery: routine clinical care	67
Table 5.1	Characteristics of the women recruited	89
Table 5.2	Postpartum characteristics of the infants and women recruited	90
Table 5.3	The mean values and standard deviation and range of maternal glucose and lactate levels in peripheral venous plasma, and various hormones and metabolites in umbilical venous cord levels of term infants at birth	91
Table 5.4	Maternal and infant factors that influence the umbilical cord venous plasma glucose level	93
Table 5.5	Maternal and infant factors that influence the umbilical cord venous plasma lactate level	94
Table 5.6	Univariate analysis model for cord glucose and lactate	146
Table 5.7	Univariate regression model for cord glucose and lactate	148

ABBREVIATIONS

ACOG	American College of Obstetricians and Gynaecologists
AD	Aldehyde dehydrogenase
ADH	Alcohol dehydrogenase
ADP	Adenosine diphosphate
4-APP	4-aminophenazone
AR	Aldose reductase
ATP	Adenosine triphosphate
BE	Base excess
BMI	Body mass index
cHCO ₃	Bicarbonate
COMT	Catechol-O-methyltransferase
C/S	Caesarean section
DA	Dopamine
2,4-DCP	2,4-dichlorophenol
DβH	Dopamine β-hydroxylase
DHPG	3-4-dihydroxyphenylglycol
DHMA	3,4-dihydroxymandelic acid
DOPA	3-4-dihydroxyphenylalanine
DOPAC	3-4-dihydroxyphenylacetic acid
DOPAL	3,4-dihydroxyphenylacetaldehyde
DOPEGAL	3,4-dihydroxyphenylglycolaldehyde
DOPET	3,4-dihydroxyphenylethanol
EMT	Extraneuronal monoamine transporter
EPI	Epinephrine

IX

ER	Endoplasmic reticulum
G-6-P	Glucose-6-phosphate
G-6-Pase	Glucose-6-phosphatase
G-6-PC1	G-6-Pase catalytic subunit
G-6-PDH	Glucose-6-phosphate dehydrogenase
Gluconate-6-P	Gluconate-6-phosphate
GSD	Glycogen storage disease
11 β -HSD-2	11 β -hydroxysteroid dehydrogenase type 2
hGH	Human growth hormone
HPLC	High performance liquid chromatography
HVA	Homovanilic acid
LDH	Lactic dehydrogenase
MHPG	3-methoxy-4-hydroxyphenylglycol
MAO	Monoamine oxidase
MOPET	3-methoxy-4-hydroxyphenylethanol
MRI	Magnetic resonance imaging
NAD	Nicotinamide dinucleotide
NADP	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
NE	Norepinephrine
NET	Norepinephrine transporter
NICE	National Institute for Clinical Excellence
OFC	Occipital-frontal head circumference
PNMT	Phenoxyethanolamine N-methyltransferase
RCOG	Royal College of Obstetricians and Gynaecologists

SCBU	Special care baby unit
SD	Standard deviation
SGA	Small for gestational age
SULT	Sulfotransferase
T ₄	Thyroxine
T ₃	Triiodothyronine
UTI	Urinary tract infection
VMA	Vanillylmandelic acid

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DECLARATION

I declare that I am the sole author of this thesis and that all references cited have been personally consulted by me, and the work of which this thesis is a record has been done by myself. This thesis has not been previously accepted for a higher degree.

Dr. Daisy Ko Ming Koh

We confirm that Daisy Ko Ming Koh has fulfilled the conditions of University of Dundee and is thereby qualified to submit this thesis in application for the degree of Doctor of Medicine.

Dr. Fiona LR Williams

Professor Robert Hume

ABSTRACT

Background The postnatal failures of expression of the hepatic glucose-6-phosphatase system suggest there are developmental deficiencies in the mechanism to 'switch-on' this key enzyme of gluconeogenesis at the time of birth in both preterm and term infants. The evidence for hormonal regulation of this critical enzyme system in animal studies, in adult humans, and studies of cell lines make the possible failure of hormonal control around the time of birth an important hypothesis to test, but before this can be done, further studies of perinatal metabolism and its hormonal control need to be undertaken.

Objective To describe the hormonal and metabolic profiles of 'unstressed' term infants delivered by elective caesarean section.

Methods One hundred and fifty three women who had an elective caesarean section for a singleton pregnancy at term in Ninewells Hospital and Medical School, Dundee were recruited between July 2004 and April 2006. Maternal venous blood was taken for glucose and lactate estimations. Umbilical venous cord blood was obtained for the measurement of glucose, lactate, 3-hydroxybutyrate, free fatty acids, amino acids (alanine, arginine, citrulline, cystine, GABA, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine ornithine, phenylalanine, serine, taurine, threonine, tyrosine and valine), insulin, glucagon, human growth hormone, cortisol, catechols (EPI, NE, DA, DOPA, DHPG, DOPAC) and their sulfated conjugates (EPI sulfate, NE sulfate, DA sulfate, DOPA sulfate, DHPG sulfate, DOPAC sulfate), and blood gas and acid-base profiles.

Results The mean maternal glucose and lactate levels were 4.28 mmol/l and 1.8 mmol/l respectively. Three infants were hypoglycaemic with umbilical blood glucose levels of less than 2.6 mmol/l. The mean infant glucose and lactate levels were as expected for full term infants at 3.3 mmol/l and 2.2 mmol/l respectively. High mean levels of insulin and catechols were noted at birth. There was a significant positive association between umbilical venous cord glucose levels and maternal venous glucose levels ($p < 0.001$) but no association of umbilical venous cord glucose levels with amino acids, insulin, glucagon, human growth hormone, cortisol and catechols.

Conclusions This extensive data set of levels of metabolites and hormones in infants at birth acts as a reference source and will be valuable for evaluating any effects of antenatal or intrapartum factors on the hormonal and metabolic profiles of infants at birth as well as to investigate the mechanisms to 'switch-on' the key enzyme glucose-6-phosphatase.

CHAPTER 1

INTRODUCTION

1.1 CEREBRAL DAMAGE AND HYPOGLYCAEMIA

Glucose is the primary essential fuel for the brain. The adult brain has high energy requirements, estimated at approximately 1.0mg/kg/min (5.6 mmol/kg/min) glucose, which corresponds to approximately 100g (555 mmol) over 24 hours (Bolli and Fanelli, 1999). The brain does not make sufficient glucose for its own needs; it is dependent on a constant supply of glucose from blood.

The brain has an amazing ability to regulate cerebral blood flow during periods of hypoglycaemia. Anwar and Vannucci demonstrated significant increases in cerebral blood flow and a positive correlation between mean arterial blood pressure and cerebral cortical blood flow, suggesting a loss of cerebral blood flow autoregulation, in newborn dogs when blood glucose concentrations fall to around 2.2 mmol/l (Anwar and Vannucci, 1988). Glucose enters human brain cells by facilitated diffusion mediated by specific glucose transporters, GLUT1 and GLUT3; both of these are present in the brains of both preterm and term infants (Mantych *et al.*, 1993).

Newborn infants remain asymptomatic even at a low blood glucose level that would cause severe symptoms in adults (Griffiths, 1968). Such asymptomatic hypoglycaemia suggests that newborn infants may be using alternative metabolic substrates for brain metabolism. Bolli and Fanelli

reported that the adult brain cannot oxidize substrates other than glucose acutely during hypoglycaemia (Bolli and Fanelli, 1999). In contrast, studies show that fetal and neonatal brains have the capacity to use alternative substrates especially lactic acid, ketone bodies (e.g. 3-hydroxybutyrate), free fatty acids (FFA) and certain amino acids (Adam *et al.*, 1975; Persson *et al.*, 1972; Vannucci and Vannucci, 2000). A study on the fasting human newborn showed that glucose oxidation can only supply up to 70% of the estimated brain metabolic requirements which suggests that alternative brain fuels are important for newborn brain metabolism during fasting (Denne and Kalhan, 1986). A tracer study has confirmed active consumption of ketone bodies and ketogenesis even in regularly fed human newborns (Boungneres *et al.*, 1986). Studies in newborn dogs have shown that lactic acid is an important alternative substrate for the brain during hypoglycaemia and it can support up to 60% of the total brain energy metabolism (Hernández *et al.*, 1980; Hellmann *et al.*, 1982). Lactate has also been shown to be an important cerebral substrate for children with glucose-6-phosphatase deficiency, a condition where children have a problem generating glucose (Fernandes *et al.*, 1984). These alternative brain fuels may be protective of brain integrity in the event of hypoglycaemia.

Glucose and oxygen are required for optimal energy generation and defects in the supply of either can cause cerebral impairment in infants and children. Oxygen deprivation is a well established cause of brain damage during labour and delivery, but glucose deprivation during labour and delivery has not been investigated as a cause of brain damage.

1.1.1 Neuropathological consequences of hypoglycaemia

Brain damage caused by hypoglycaemia is a result of a sequence of events which include activation of neuronal glutamate receptors and release of zinc, leading to production of reactive oxygen species from mitochondria and activation of poly (ADP-ribose) polymerase-1 (Suh *et al.*, 2007). The pattern and distribution of neural damage from hypoxic-ischaemic brain injury are different from hypoglycaemic injury. Hypoxic-ischaemic injury is characterised by infarction, a predominance of boundary zone injury and greater involvement of the deeper cortical layers (Williams, 2005). In contrast, following hypoglycaemia, the predominant features are diffuse damage of the cortical and subcortical white matter with the parietal and occipital lobes affected most severely (Anderson *et al.*, 1967; Banker, 1967). With the development of imaging techniques, Spar and colleagues described the first case of extensive occipital cortical loss from neonatal hypoglycaemia on computerised tomography and magnetic resonance imaging (MRI) studies (Spar *et al.*, 1994). Subsequent studies also confirmed a similar pattern of injury with severe hypoglycaemia (Barkovich *et al.*, 1998; Filan *et al.*, 2006). This pattern of injury is also seen in neonates with transient hypoglycaemia i.e. symptomatic hypoglycaemia with a glucose level less than 2.5 mmol/l but the symptoms disappear after restoration of normal blood glucose levels (Kinnala *et al.*, 1999). Kinnala and colleagues described four infants who suffered from transient hypoglycaemia with patchy hyperintensity lesions, either in the occipital periventricular white matter, or the thalamus on T1 weight images on MRI, but the lesions were transient and most of them were absent two months later; the clinical relevance of these abnormal

findings remains unknown (Kinnala *et al.*, 1999). As yet, there is still no clear explanation of why the parietal and occipital lobes are most severely affected. However, it has been suggested that it may be related to the development of receptors for excitatory amino acids which can be stimulated by aspartate and glutamate and result in selective neuronal necrosis (Barkovich *et al.*, 1998).

1.2 DEFINITION OF HYPOGLYCAEMIA

The definition of what constitutes hypoglycaemic blood glucose levels in infants remains contentious (e.g. Schwartz, 1997; Cornblath *et al.*, 2000). A large survey of 508 neonatal paediatricians in the United Kingdom and Australia, and a review of fourteen neonatal textbooks in 1992 showed that there is a wide range in the levels of blood glucose that are used to define hypoglycaemia with values from less than 1 mmol/l to 4 mmol/l (Koh and Vong, 1996). Furthermore different values were suggested for term and for premature/small for gestational age infants (Koh and Vong, 1996)

Four approaches have been used to define hypoglycaemia in the neonate: clinical, statistical, physiological and neurodevelopmental.

1.2.1 Clinical definition of hypoglycaemia

The diagnosis of hypoglycaemia based on clinical manifestation was first introduced by Whipple and Fratz in 1935 using three criteria - known as the Whipple triad, which required to be fulfilled (Whipple and Fratz, 1935). First, there had to be evident signs and symptoms of hypoglycaemia; secondly, an

accurate method had to be available to measure (low) glucose levels; thirdly, there should be a prompt resolution of clinical signs once normoglycaemia was re-established. However there are difficulties with this clinical approach to define hypoglycaemia. For example, the symptomatic response of the neonate to low blood glucose is variable with clinical features which are, in general, non-specific and which include pallor, feeding difficulties, hypotonia, tachypnoea, abnormal cry, jitteriness, apnoea, irritability, coma and convulsions (e.g. Hume, 1998). This lack of symptomatic specificity can make the interpretation of biochemical hypoglycaemia difficult. This is particularly so in the intensively cared for infant where concurrent pathologies cause the same pattern of clinical features or where symptomatic responses may be depressed with therapeutic muscle paralysis, severe birth asphyxia or extreme prematurity. Hypoglycaemia may be intermittent and it is important to check blood glucose levels at times of vague but suspicious symptoms (e.g. Hume, 1998). Some otherwise well infants with low blood glucose levels are asymptomatic suggesting that alternative metabolic substrates are available for brain metabolism and such infants in general have a more favourable prognosis (Cornblath and Schwartz, 1976).

1.2.2 Statistical definition

Hypoglycaemia has been defined by using a level of more than two standard deviations (SD) below the mean obtained from populations of full term and low birth-weight infants (Pildes *et al.*, 1967; Heck and Erenberg, 1987). However, the findings of this statistical approach reflects the population distribution of values - both physiological and potentially pathological, and this has generated

anomalies, for instance, where hypoglycaemia was defined as a blood glucose concentration for a particular population. For example, hypoglycaemia in the preterm or low birthweight infant was defined as less than 1.1 mmol/l during the first week of life, whereas, in full-term infants, the corresponding value was less than 1.7 mmol/l for the first 72 h of life with a value of less than 2.2 mmol/l after 72 h of age (Cornblath and Schwartz, 1976). These criteria imply that preterm infants compared to term are less prone to neurological impairment secondary to hypoglycaemia and that resistance to hypoglycaemia is greatest in the immediate postpartum period. A more satisfactory approach may be to define significant biochemical hypoglycemia in the context of acute or chronic neurological dysfunction.

1.2.3 Physiological definition

1.2.3.1 Hormonal responses to hypoglycaemia

Blood glucose levels are regulated by hormonal mechanisms and the blood glucose reducing effect of insulin is counter-regulated by cortisol, catecholamines, human growth hormone (hGH) and glucagon (Bolli and Fanelli, 1999). As blood glucose levels fall, the counter-regulatory hormones increase. For example in adults, the blood glucose threshold level for initiating the response of cortisol is 3.2 mmol/l (Schwartz *et al.*, 1987); a cortisol response at this blood glucose level may indicate a physiological requirement to maintain blood glucose levels above 3.2 mmol/l and indicate the lower limit of normoglycaemia. In infants and children more than six months old, who were spontaneously hypoglycaemic (glucose level less than 2.5 mmol/l), a similar glycaemic threshold for cortisol was noted (Crofton and Midgley, 2004).

However, poor cortisol response to hypoglycaemia was found in infants who were less than three months old and it has been suggested that this could be due to the relatively low glucose levels at birth resulting in poor hypothalamic-pituitary response to hypoglycaemia (Crofton and Midgley, 2004). By contrast, studies of metabolic and hormonal responses to hypoglycaemia in preterm and small for dates term infants demonstrated an elevated cortisol response to a relatively short duration of hypoglycaemia (Hume *et al.*, 1999; Jackson *et al.*, 2004). There remains a lack of consensus in the limited studies available in the newborn to define a blood glucose level that defines hypoglycaemia in terms of the threshold level for a cortisol response.

Definitions of infant hypoglycaemia based on a blood glucose level that induces a counter-regulatory response in hormones other than cortisol are also problematic. In adults, a blood glucose level of 3.7 mmol/l is the threshold level for hGH response (Schwartz *et al.*, 1987) and indirect evidence has suggested that older infants and children more than six months old have a similar hGH threshold but their hGH response to spontaneous hypoglycaemia is reduced in magnitude (Crofton and Midgley, 2004). However this is not evident in preterm infants as hGH levels were similar in fasted normoglycaemic and hypoglycaemic preterm infants prior to discharge from hospital (Hume *et al.*, 1999) and also in less than three months old infants who were normo- or hypoglycaemic at sampling (Crofton and Midgley, 2004).

In human adults, glucagon and epinephrine (EPI) play an important role in increasing glucose levels during acute (Cryer *et al.*, 1984) as well as in

prolonged hypoglycaemia (Bolli *et al.*, 1984). However, in preterm infants at the time of discharge from hospital or small for dates term infants studied in the neonatal period, there was a lack of glucagon response to hypoglycaemia (Hume *et al.*, 1999; Jackson *et al.*, 2004). The counter-regulatory hormonal response to hypoglycaemia in early infancy appears to be limited to elevations of cortisol and EPI (Jackson *et al.*, 2004) and there is insufficient current data to define a blood glucose threshold level to initiate these responses.

1.2.3.2 Neurophysiological definition

In 1988, Koh and colleagues demonstrated that acute cerebral dysfunction (auditory and somatosensory evoked potentials) occurred in ten of eleven children when blood glucose levels fell below 2.6 mmol/l and when 50% were asymptomatic (Koh *et al.*, 1988). They recommended that blood glucose concentration in children should be maintained above 2.6 mmol/l to ensure normal neural function, regardless of the presence or absence of abnormal clinical signs. The critical level of 2.6 mmol/l was supported in a further study of cerebral blood flow, which was found to be increased in hypoglycaemic preterm infants when the blood glucose level was less than 2.5 mmol/l although the neonates had no clinical signs of hypoglycaemia (Pryds *et al.*, 1988; Pryds *et al.*, 1990). In contrast, a subsequent small study found no correlation between glucose concentrations, ranging from 1.39 to 6.83 mmol/l, and the brainstem auditory evoked response; they concluded that tighter clinical control of glucose homeostasis is probably not required in the neonatal period (Cowett *et al.*, 1997). The findings from these studies are not clear; they

were small studies and underpowered to show definitely whether there is a significant correlation with brain damage.

1.2.4 Neurodevelopmental approach

Studies have shown that prolonged or recurrent neonatal hypoglycaemia may lead to permanent neurological damage in term infants (Aynsley-Green and Soltesz, 1985; Cowett, 1999). Lucas and colleagues carried out a detailed multicentre study of 661 preterm infants who weighed less than 1850g at birth and evaluated the neurodevelopment outcomes relative to hypoglycaemia at 18 months of age (Lucas *et al.*, 1988). They found that two-thirds of the neonates had blood glucose levels less than 2.6 mmol/l for a period ranging from 3 to 30 days. If hypoglycaemia existed for five or more separate days, the Bayley motor and mental developmental scores were significantly reduced at 18 months (after adjustment for identifiable confounders) and the incidence of the neurodevelopmental impairment was increased by a factor of 3.5. This study only included preterm infants who weighed less than 1850g. Therefore, the results may not be applicable to term infants. Subsequently, Duvanel and colleagues reported that recurrent episodes of hypoglycaemia with a blood glucose level below 2.6 mmol/l were strongly correlated with reduced head circumferences at 18 months and lower psychometric scores at five years of age in small-for gestational age preterm neonates (Duvanel *et al.*, 1999). Head circumference at birth has been suggested to be predictive of cognitive function in childhood. In a cohort of 249 very low birth weight children, those with a normal head circumference at birth have better cognitive function when compared to

those whose head size was more than two SDs below the mean at eight years of age (Hack *et al.*, 1991). Another study carried out by Peterson and colleagues looking at the effect of head circumferences on academic achievement and behaviour in a cohort of 128 children who weighed less than 1500g at birth, found that a head circumference more than two SDs below the mean was associated with poorer IQ, perceptual motor skills, academic achievement and adaptive behaviour than those whose head size was normal (Peterson *et al.*, 2006)

None of these approaches to the definition of hypoglycaemia has been entirely satisfactory. A recent review suggested that it is time to move away from a single threshold definition of 'hypoglycaemia' towards a more flexible approach which takes into account not only the plasma glucose concentration but also the presence of risk factors and the clinical state of the infant (Williams, 2005).

1.2.5 Conclusion

There is no universal definition of hypoglycaemia in infants and hence an operational threshold for hypoglycaemia which is defined as "*the concentration of plasma or whole blood glucose at which clinicians should consider intervention, based on the evidence currently available from the literature*" has been suggested (Cornblath *et al.*, 2000). Currently, the general consensus from the neonatologists in the United Kingdom for the operational threshold for hypoglycaemia is 2.6mmol/l.

1.3 FETAL METABOLISM DURING DELIVERY

In labour, the oxygen level in the fetal blood decreases during a normal uterine contraction and for a period the fetus will not metabolise glucose completely to carbon dioxide but instead to acidic metabolic intermediates such as lactate. This only partially releases the potential energy of glucose and depletes fetal liver glucose reserves (which is in the form of glycogen). With the relaxation between contractions, restitution of gas exchange occurs but it is not possible to restore the depleting hepatic glycogen stores. Anaerobic metabolism is an inefficient use of glucose and may result in depletion of reserves and subsequent lowering of fetal blood glucose levels. The fetus is dependent on restoration of blood glucose levels from the maternal circulation which is re-established between contractions. This restoration may be compromised if the mother has sub-optimal blood glucose levels. Intrauterine growth restriction fetuses are particularly at risk of hypoglycaemia during labour due to limited hepatic glycogen storage (Shelley and Neligan, 1966). Similarly, preterm fetuses are at risk too as hepatic glycogen only accumulates in the human fetus in the late third trimester (Shelley, 1964). During labour, lactic acid accumulates in the fetus and eventually lactic acidaemia develops. Carbon dioxide diffuses rapidly across the placenta but a fixed acid such as lactic acid diffuses slowly across the placenta into the maternal circulation (Gilstrap, 1999a). Accumulation of lactate in the brain tissue can lead to oedema and necrosis (Gilstrap, 1999a). Carbon dioxide levels in general are higher in the fetus than in the infant. In solution, carbon dioxide is acidic and this combined with lactate means that the fetus during delivery is in an even more acidic

environment. The degree of acidity is routinely assessed by measuring cord pH, and oxygen and carbon dioxide levels in the blood.

1.3.1 Perinatal glucose homeostasis

In-utero the human fetus is dependent upon a maternal supply of glucose which is transported by facilitated diffusion across the placenta; there is no significant production of glucose by fetus *in-utero* (Kalhan *et al.*, 1979). It has been shown in fetal sheep that the rate of placental glucose transfer to the fetus is enhanced by increasing the maternal-fetal glucose concentration gradient (Hay and Mezmarich, 1989). The maternal-fetal glucose concentration gradient is especially important in the intrauterine growth restriction fetus where there is a reduction in placental glucose transport capacity, and hence, high maternal-fetal glucose concentration gradients increase the maternal-fetal glucose transfer (Hay, 2006).

Placental and fetal glucose consumption has a major influence on the maternal-fetal glucose concentration gradient and hence the transfer of glucose from the maternal circulation to the fetus. The placenta has a high glucose consumption, using approximately 75% of the glucose supply to the utero-placental unit (Sparks *et al.*, 1983). In the fetal sheep, placental glucose consumption is directly related to fetal arterial plasma glucose levels and is independent of maternal glucose levels (Hay *et al.*, 1990). The fetal side of the placenta has an eightfold greater glucose transport capacity compared to the maternal side, which means fetal blood glucose concentration has a great influence on the glucose metabolism in the

placenta (Hay, 2006). With advancing gestation, the placental glucose transfer to the fetus increases by increasing the placental glucose transport capacity as well as increasing the maternal-fetal glucose concentration gradient (Hay, 2006).

Immediately after delivery, with the ligation of the umbilical cord, the fetal glucose concentration falls, and thereafter stabilisation is dependent on the mobilisation of hepatic glycogen stores (Shelley and Neligan, 1966), maturation and activation of key gluconeogenic and glycogenolytic enzymes (Girard, 1986), an integrated metabolic and hormonal response (Girard, 1986), and the initiation of milk feeding.

1.3.2 Hepatic glycogen stores

Fetal hepatic glycogen stores rise appreciably from 36 weeks gestation but the level falls considerably soon after birth (Shelley and Neligan, 1966). Hepatic glycogen stores are lower in preterm infants and infants who have suffered severe respiratory distress compared to normal term infants (Shelley, 1964). In newborn term infants, the hepatic glycogen concentration falls within 24 hours to 10% of that at birth, but in premature infants, the hepatic glycogen level becomes nearly depleted within 12 hours of birth (Shelley and Neligan, 1966).

1.3.3 Hepatic microsomal glucose-6-phosphatase system

The induction of the key regulatory enzymes of gluconeogenesis and glycogenolysis e.g. hepatic glucose-6-phosphatase (G-6-Pase), must take

place post-delivery to allow liver glucose production and the maintenance of blood glucose levels. G-6-Pase catalyses the hydrolysis of glucose-6-phosphate (G-6-P) to glucose and phosphate, which is the final step in the metabolic pathways by which liver produces glucose (Ganong, 1995a).

The G-6-Pase system consists of at least two integral membrane proteins, G-6-P transporter and the G-6-Pase catalytic subunit (G-6-PC1) (Arion *et al.*, 1975). The G-6-PC1 is an endoplasmic reticulum (ER) membrane protein and its active site is inside the lumen of the ER (Burchell *et al.*, 1990). Therefore for hydrolysis, G-6-P needs to be transported across the ER membrane from the cytoplasm to reach the G-6-PC1. The products, glucose and phosphate, also need to be shuttled from the lumen of ER into the cytoplasm for release into the circulation. Arion and colleagues proposed a model of the G-6-Pase system, which consisted of separate microsomal transporters, T1 for G-6-P, T2 for phosphate and T3 for glucose (Arion *et al.*, 1975). To date, T1 has been identified (Chen and Burchell, 1995) but T2 and T3 have yet to be characterised. From the cytoplasm, glucose is released into the bloodstream via the facilitative plasma membrane glucose transport protein, GLUT 2 (Chen and Burchell, 1995).

Any genetic or regulatory deficiency of the different protein component parts of the G-6-Pase system results in abnormally low liver glucose production with the potential to cause severe episodes of fasting hypoglycaemia (Chen and Burchell, 1995). Type I glycogen storage disease (GSD) is an autosomal recessive genetic disorder of the hepatic microsomal G-6-Pase system (Chen

and Burchell, 1995). The condition may present in the newborn period with hypoglycaemia and/or lactic acidosis but more characteristically presents at three to four months of age with hepatomegaly and fasting hypoglycaemia with seizures (Chen and Burchell, 1995). However some individuals with Type I GSD remain undiagnosed until adulthood (Burchell *et al.*, 1987). Long term complications include delayed puberty, reduced adult height, gout, renal disease, pancreatitis, premature arteriosclerosis, hepatic adenomas and hypertension (Chen and Burchell, 1995).

1.3.3.1 The ontogeny of the human hepatic microsomal glucose-6-phosphatase

Hepatic G-6-Pase enzyme activity first appears as early as eleven weeks gestation in humans and the level remains low before birth, gradually increasing to about 10% of adult values by term (Burchell *et al.*, 1990). However after birth, the activity rises rapidly in term infants reaching adult values by about three to four days of age and the increase of the enzyme activity is equalled by an increase in the concentration of the G-6-Pase enzyme protein (Burchell *et al.*, 1990). After the first week, the level remains constant until adulthood (Burchell *et al.*, 1990). Burchell and colleagues also showed that T1 developed at the same time and rate as the G-6-PC1 whereas T2 developed later in gestation, between 20 and 24 weeks gestation, which is when the human liver production of glucose reported reaches full activity (Burchell *et al.*, 1990).

The ontogeny of G-6-Pase enzyme activity in preterm infants and term infants is different. Hume and Burchell studied the hepatic microsomal G-6-Pase enzyme in liver samples from 76 preterm infants between 3 and 360 days of age and demonstrated that most had G-6-Pase activities which were below the extreme lower limit of the normal term infant range and which could render these infants at risk of repeated hypoglycaemic episodes and brain damage (Hume and Burchell, 1993). In a subsequent study, they found that 18% of a cohort of 79 consecutively delivered preterm infants who were ready for discharge home failed to maintain normoglycaemia, suggesting a significant number of preterm infants are at risk of hypoglycaemia at home if a feed is omitted or delayed (Hume *et al.*, 1999).

1.3.3.2 Perinatal factors and the regulation of glucose-6-phosphatase gene expression

Since the 1950s, animal studies have shown that the activity of G-6-Pase is influenced by various hormones and metabolites. For example, G-6-Pase enzyme activity (Ashmore *et al.*, 1954; Burchell and Cain, 1985), protein levels (Burchell and Cain, 1985) and mRNA levels (Liu *et al.*, 1994) all increase in diabetic rats. Starvation also causes a two to threefold increase in hepatic G-6-Pase activity (Ashmore *et al.*, 1954). However, the activity is depressed by administration of insulin in diabetic (Ashmore *et al.*, 1954) as well as normal state rats (Nordlie *et al.*, 1965a). Studies in rat hepatoma cells have also shown that insulin decreases G-6-Pase mRNA (Argaud *et al.*, 1996). Glucocorticoids *in vivo* increases hepatic G-6-Pase activity levels by about 40% in the adrenalectomized rats (Nordlie *et al.*, 1965b). Glucocorticoids also

stimulate the transcription of the gene encoding the catalytic subunit of the G-6-Pase (Streeper *et al.*, 1997; Hiraiwa and Chou, 2001) whereas insulin strongly inhibits both basal G-6-Pase gene transcription and the stimulatory effect of glucocorticoids (Streeper *et al.*, 1997). Argaud and colleagues have demonstrated in rats that glucose causes a 20-fold increase of the level of G-6-Pase mRNA transcripts in cultured hepatocytes and a threefold increase in hepatoma cells (Argaud *et al.*, 1997). The transcription of the human G-6-Pase gene has also been shown to be down regulated by insulin (Schmoll, 1996) and up regulated to a lesser extent by glucose, cyclic AMP and dexamethasone (Schmoll *et al.*, 1999).

However the response of post-mortem hepatic G-6-Pase activity in 36 preterm infants who had therapeutic administration of insulin during the postnatal period yielded surprising results (Burchell *et al.*, 2000). Instead of a negative correlation with insulin levels, the levels of hepatic G-6-Pase activity was positively correlated with the amount of insulin prescribed whilst the infant was alive. Currently, the understanding of the *in vivo* regulation of human hepatic G-6-Pase in preterm infants is rudimentary and based on a cohort of infants who have different basal levels of a variety of hormones and metabolites. Another study carried out by Hume and colleagues on 45 preterm infants demonstrated that infants with severe respiratory distress and/or those exposed to pathogenic bacterial infections, whether *in-utero* or the early postnatal period, have higher levels of G-6-Pase enzyme activity (Hume *et al.*, 2000). Hume and colleagues found that the perinatal factors that are associated with the changes in hepatic G-6-Pase enzyme activity were the

presence of pathologic bacteria mainly *Streptococcus agalactia* isolated from maternal high vaginal swabs taken during labour, the duration of prenatal exposure to ritrodine, and delivery by caesarean section.

In the study of fetal sheep, the rise of G-6-Pase and phosphoenolpyruvate carboxykinase enzyme activities in the liver and kidney were prevented when the rise in plasma triiodothyronine (T_3) was prevented by thyroidectomy prior to delivery (Forhead *et al.*, 2003). This result suggests that thyroid hormones play an important part in the activation of gluconeogenesis pathway in the fetal life.

1.3.4 Perinatal hormonal and metabolic responses to extrauterine life

Within minutes of delivery, catecholamines rise three to tenfold in the human (Sperling *et al.*, 1984). Glucagon also increases three to fivefold and remains elevated throughout the first day of life in the human fetus (Sperling *et al.*, 1974). Simultaneously, hGH and cortisol rise with a concomitant fall in insulin secretion in the human fetus (Menon and Sperling, 1988). There is also a marked postnatal surge of thyroid stimulating hormones at about 30 minutes of age secondary to the exposure of the infants to cold in the extrauterine environment. This in turn leads to secretion of thyroidal thyroxine (T_4) and T_3 which peak at between 24 to 36 hours of age (Fisher, 1996).

Over the first few days of life, with increases in enteral intake and with continued maturation of hepatic gluconeogenesis (Lucas *et al.*, 1978), blood glucose levels stabilise. Transient disturbances in neonatal glucose

homeostasis are common during this time, especially where metabolic reserves are low as in prematurity and intra-uterine growth retardation or, where expenditure of energy is high as seen, for example, with sepsis, birth asphyxia and hypothermia (Lyall *et al.*, 1994; Hume *et al.*, 1998). Preterm infants have lower hepatic glycogen reserves (Shelley, 1964), lower activities of key gluconeogenic enzymes (Burchell *et al.*, 1990), an initially limited hormonal response (Lucas *et al.*, 1978) and, even where regimens of postnatal care were similar to those of term infants (i.e. oral milk feeds), blood glucose values were lower and the postnatal rise was slower (Smallpeice and Davies, 1964).

1.3.4.1 The physiological role of amino acids in the perinatal period

Amino acids are important substrates for protein synthesis and oxidation during intrauterine life. The plasma amino acid concentrations do not change significantly from second to third trimester during pregnancy (Cetin *et al.*, 1990). It has been well established that plasma amino acid concentrations in the fetus at mid-gestation as well as at term (Cetin *et al.*, 1990), are higher than those in the mother which suggests that amino acids are actively transported from the mother to the fetus (Galan *et al.*, 2009). The transport is regulated by specific amino acid transporters which have been found on the microvillous (Johnson and Smith, 1988) and basal membranes of the syncytiotrophoblastic cells of human placenta (Hoeltzli and Smith, 1989).

There are a number of factors which regulate the activity of the transport system. The capacity of amino acid transport systems gradually increases with gestation to support the increasing nutrient demands of the developing

fetus (Mahendran *et al.*, 1994). Increasing maternal amino acid concentration also increases the transport capacity and ultimately the amino acid concentration in the fetus. This has been demonstrated in sheep for alanine (Timmerman *et al.*, 1998) and lysine (Wilkes *et al.*, 2003). However not all the amino acid levels will increase in the fetus when a mother is given a large number of amino acids, due to the competition for the shared transporters (Józwik *et al.*, 2001). Animal studies have demonstrated that the amino acid transport across the placenta is reduced in the growth restricted fetuses and logically this would suggest a lower amino acid concentration in the growth restricted fetus compared to the normal well grown fetus. However there is conflicting evidence of this. Economides and colleagues and Cetin and colleagues have demonstrated reduced umbilical venous amino acid concentrations in the growth restricted fetus (Economides *et al.*, 1989; Cetin *et al.*, 1990) but a more recent study by Paolini and colleagues found no difference in the umbilical venous amino acid concentration in the normal and growth restricted fetuses (Paolini *et al.*, 2001). There is also a significant increase in a number of amino acids in the umbilical cord vein in pregnancies complicated with gestational diabetes mellitus, preexisting diabetes and preeclampsia (Cetin *et al.*, 2005).

1.3.4.2 The physiological role of insulin and glucagon in the perinatal period

In fetal sheep, there is a gradual increase in glucose and arginine stimulated insulin secretion, as well as basal insulin levels from mid-gestation to term (Aldoretta *et al.*, 1998). In human fetus, umbilical insulin levels obtained by

cordocentesis showed that insulin levels increased exponentially from 20 weeks to 38 weeks gestation which suggests a progressive maturation of pancreatic activity with gestation (Economides *et al.*, 1989). However the levels appear to fall after 37 weeks gestation (Godfrey *et al.*, 1996) and remain low at birth (Thomas *et al.*, 1967).

Insulin secretion can be down-regulated in chronic and constant hyperglycaemia as demonstrated in fetal sheep in mid and late gestation (Carver *et al.*, 1995; Aldoretta *et al.*, 1998). Interestingly, pulsatile hyperglycaemia can stimulate insulin secretion in fetal sheep (Carver *et al.*, 1996). Chronic hypoglycaemia can also decrease the insulin secretion response in fetal sheep and the response to hypoglycaemia can still be blunted after recovery from the hypoglycaemic episodes (Limesand and Hay, 2003). Hence, Limesand and Hay suggested that this could be the possible mechanism of developing type II diabetes in adult life in intrauterine growth restricted infants (Limesand and Hay, 2002).

Conversely, hypoinsulinaemia, which can result from a number of conditions such as pancreatic agenesis and transient diabetes can lead to intrauterine growth retardation in the fetus (Fowden, 1995). Osmanağaoğlu and colleagues have demonstrated that umbilical venous insulin levels were significantly lower in small for gestational age infants and that the levels correlated with birth weight, birth length and placental weight (Osmanağaoğlu *et al.*, 2005). Umbilical cord insulin levels were higher in infants delivered by elective caesarean section compared to infants delivered

by spontaneous vaginal delivery (Wang *et al.*, 1995, Godfrey *et al.*, 1996, Shields *et al.*, 2007). Infants whose mothers went into labour spontaneously were also found to have higher umbilical insulin levels compared with those whose mothers were induced (Godfrey *et al.*, 1996). There is conflicting evidence on the influence of gender on umbilical insulin levels. A study carried by Godfrey and colleague on 391 term infants concluded that gender does not appear to influence the levels of insulin in venous cord blood (Godfrey *et al.*, 1996) but the more recent study by Shields and colleagues suggest that female infants have higher umbilical venous insulin levels compared to male infants (Shields *et al.*, 2007).

There is paucity of evidence on the differences on insulin levels in the umbilical vein and artery. Thomas and colleagues studied the umbilical cord insulin levels in 14 appropriate weight-for-gestation term infants, concluded that the levels were similar in the umbilical artery and vein (Thomas *et al.*, 1967). Similarly there is paucity of evidence on the umbilical cord glucagon levels at birth in the human fetus. At term, umbilical venous and arterial glucagon levels in the human fetus are similar to maternal levels (Milner *et al.*, 1973), but the level rises three to fivefold acutely and remains elevated throughout the first day of life in the human infant (Sperling *et al.*, 1974). Simultaneously, insulin secretion falls in the human fetus after delivery (Menon and Sperling, 1988).

1.3.4.3 The physiological role of cortisol in the perinatal period

In vivo studies measuring serum cortisol levels by cordocentesis in the fetus from mid-gestation showed that the levels remain constant between 18 and

36 weeks gestation (Economides *et al.*, 1988) and thereafter the level gradually increases (Oh *et al.*, 2006). Serón-Ferré and colleagues investigated umbilical cord cortisol levels of 57 term infants delivered by elective caesarean section over a 24 hour period and concluded that there is a 24-hour rhythm of cortisol secretion from the adrenal gland in the fetus at term (Serón-Ferré *et al.*, 2001). However there is equally substantial evidence that there is no diurnal variation until about three months postnatal age (Price *et al.*, 1983; Spangler, 1991). Cortisol levels are transiently elevated at birth in term infants delivered vaginally and decrease to lower and stable levels by 24 hours (Kraiem *et al.*, 1985).

Fetal cortisol levels are linked to antenatal or intrapartum stressful events, spontaneous onset of labour and mode of delivery. *In vivo* studies looking at human fetal stress responses to invasive stimuli between 17 and 35 weeks gestation showed that an elevated plasma fetal cortisol levels, independent of the maternal responses, to transfusion via the intrahepatic vein (Giannakouloupoulos *et al.*, 1999; Gitau *et al.*, 2001). Umbilical cord cortisol levels were also found to be elevated in associated with spontaneous onset of labour (Leong and Murphy, 1976; Mears *et al.*, 2004). It is well established that infants delivered by elective caesarean section have the lowest umbilical cord cortisol levels compared to other mode of deliveries (Mears *et al.*, 2004; Miller *et al.*, 2005; Vogl *et al.*, 2006).

Many studies have used mixed or venous cord blood. The largest study to date is by Garagorri and colleague looking at the umbilical cord cortisol

levels in 138 infants delivered by spontaneous vaginal deliveries (Garagorri *et al*, 2008). Unfortunately there was no detail on whether the sample was taken from umbilical vein or artery recorded. A study by Gitau and colleagues which looked at the cortisol levels in both umbilical cords arterial and venous found that plasma cortisol levels were significantly higher in the umbilical cord artery than in the umbilical cord vein (Gitau *et al*, 2001). They also showed that there was a significant high correlation between umbilical cord arterial and venous cortisol levels.

Gluconeogenesis has been demonstrated in fetal sheep during late gestation with a concomitant rise in cortisol and catecholamines (Fowden *et al.*, 1998). Cortisol plays an important role in the regulation of hepatic glycogen synthesis in the fetus. Studies in fetal rats have shown that cortisol increases glycogen synthetase activity and thus glycogen synthesis (Monder and Coufalik, 1972). Cortisol also enhances hepatic G-6-Pase activity and the removal of the pituitary gland results in low levels of fetal ovine plasma cortisol, and hepatic and renal G-6-Pase activity (Fowden *et al.*, 1990). Besides this, cortisol plays an important role in promoting the maturation of organ systems including lung, thyroid, pancreas and small intestine (Liggins, 1976). In the 1960s, Liggins and colleagues showed that cortisol secretion by the fetal adrenal cortex in sheep plays a critical role in the process of triggering a cascade of events leading to parturition (Liggins, 1976). However in the human, cortisol does not appear to be involved in the regulation of parturition (Ohrlander *et al.*, 1976; Sybulski and Maughan, 1976).

After delivery, cortisol plays an important role in the hormonal counter-regulation of glucose homeostasis, particularly in preterm infants. A study that investigated counter-regulatory hormonal reactions to hypoglycaemia in small for gestational age term infants showed that infants with severe and persistent hypoglycaemia had elevated cortisol and EPI, but not glucagon or hGH levels (Jackson *et al.*, 2004). Preterm and small for gestational age term infants thus appear to display different cortisol responses when exposed to repeated hypoglycaemia compared to an adult.

1.3.4.4 The physiological role and metabolism of catecholamines in the perinatal period

At birth, there are significant transitions in many vital organ systems which are critical for effective and successful adaptation to extrauterine life. Catecholamines have been shown to play an important role in the neonatal cardiovascular, respiratory and metabolic adaptation at birth (Sperling *et al.*, 1984).

Lung inflation and oxygenation at birth results in increased pulmonary blood flow, left atrial pressure and left ventricular output (Padbury and Martinez, 1988). Adrenal catecholamines release has been suggested to further promote these transitional events by enhancing the contractility of the left ventricle (Padbury and Martinez, 1988). Impaired left ventricular contractile responses have been found in adrenalectomized newborn dogs (Erath *et al.*, 1982).

There is considerable evidence to show that high levels of endogenous catecholamines may be important for accelerating the absorption of lung fluid at birth (Faxelius *et al.*, 1983; Finley *et al.*, 1998). This clearance is mainly mediated in the alveolar epithelial cells by transepithelial sodium reabsorption through the amiloride-sensitive sodium channels (Hummler *et al.*, 1996; Finley *et al.*, 1998). Beta-agonists have been shown to increase the activity of sodium channels in the lung through a cAMP-PKA-mediated mechanism (Chen *et al.*, 2002).

It is well known that infants delivered vaginally demonstrate improved functional lung residual capacity and mean thoracic gas volume when compared with infants not exposed to labour and delivered by caesarean section (Vyas *et al.*, 1981; Lee *et al.*, 1999). Animal studies have suggested that catecholamines are important in both the synthesis and release of lung surfactant, which is essential for normal pulmonary function (Padbury *et al.*, 1987). Therefore, there is a higher occurrence of respiratory morbidity in term infants delivered by elective caesarean section (Zanardo *et al.*, 2004; Hansen *et al.*, 2008) due to the combined effect of failure to switch off lung fluid production and reduced surfactant levels.

Catecholamines also play an important role in perinatal glucose homeostasis (Sperling *et al.*, 1984). Padbury and colleagues have demonstrated a significant decrease in glucose and FFA levels in surgically adrenalectomised ovine term fetuses (Padbury *et al.*, 1987). There is evidence to show that there is an arterio-venous difference in EPI and NE in

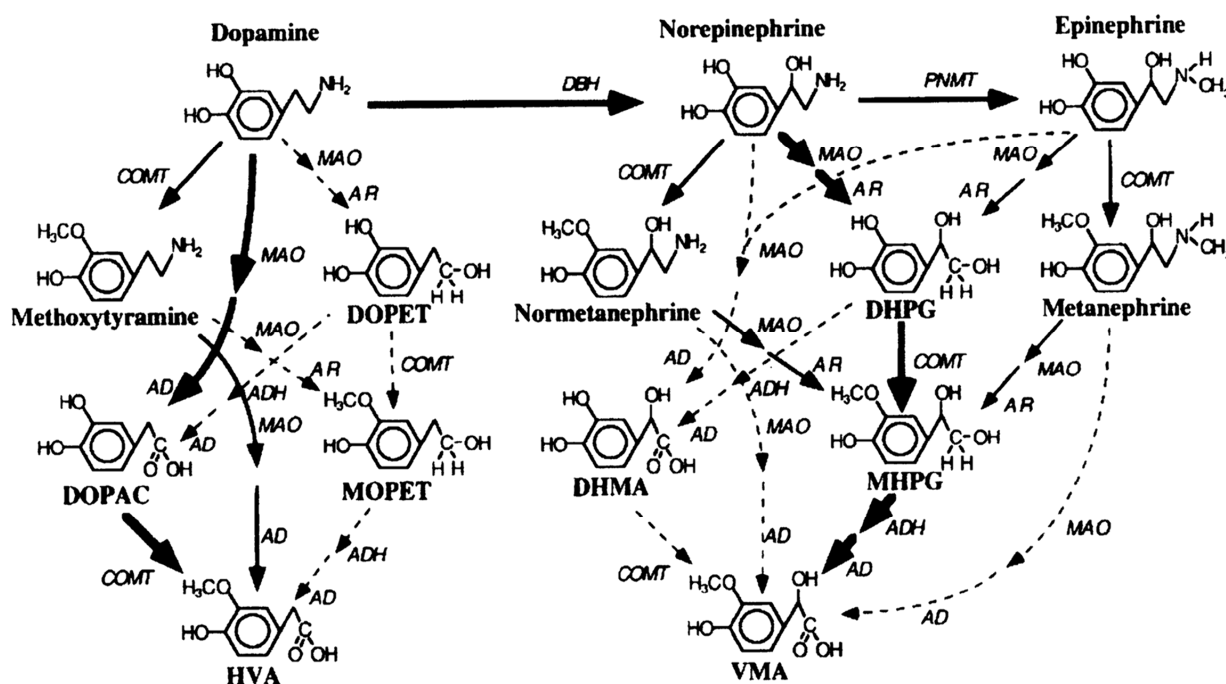
cord infant blood with venous levels lower than arterial (Eliot *et al.*, 1980, Falconer and Lake, 1982). Postnatal levels of EPI and NE are high (e.g. Eliot *et al.*, 1980) consistent with high amounts of vanillylmandelic acid (VMA) and homovanillic acid (HVA) in postnatal urine (Gabriel *et al.*, 1983). Together, these observations suggest that there is a feto-placental-maternal metabolism or transport of catechols.

Human plasma contains six readily detectable catechols. The main plasma catechols are the three catecholamines, epinephrine (EPI), norepinephrine (NE), and dopamine (DA); their precursor, 3,4-dihydroxyphenylalanine (DOPA); and their deaminated metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) from DA and 3,4-dihydroxyphenylglycol (DHPG) from NE (Eisenhofer *et al.*, 2001, Figure 1.1). The major end product of NE and EPI metabolism is 4-hydroxy-3-methoxymandelic acid (VMA) which is produced mainly (94%) in the liver by oxidation of 3-methoxy-4-hydroxyphenylglycol (MHPG) (Eisenhofer *et al.*, 2004). The major end product of DA metabolism is 3-methoxy-4-hydroxyphenyl acetic acid (HVA) which is mostly formed outside the liver by O-methylation of DOPAC or oxidative deamination of 3-methoxytyramine (Eisenhofer *et al.*, 2004). Both VMA and HVA are excreted in the urine.

Figure 1.1: Pathways of metabolism of catecholamines

(Enzymes responsible for each pathway are shown at the head of arrows.)

Adapted from Eisenhofer *et al.*, 2001 with permission).



DBH dopamine β-hydroxylase, **PNMT** phenylethanolamine N-methyltransferase, **MAO** monoamine oxidase, **COMT** catechol-O-methyltransferase, **AR** aldose reductase, **AD** aldehyde dehydrogenase, **ADH** alcohol dehydrogenase, **DOPET** 3,4-dihydroxyphenylethanol, **DOPAC** 3,4-dihydroxyphenylacetic acid, **MOPET** 3-methoxy-4-hydroxyphenylethanol, **HVA** homovanillic acid, **DHPG** 3,4-dihydroxyphenylglycol, **DHMA** 3,4-dihydroxymandelic acid, **MHPG** 3-methoxy-4-hydroxyphenylglycol, **VMA**, vanillylmandelic acid

The mechanisms for clearance of the high fetal production rate of catecholamines may be critical for fetal homeostasis. As EPI, NE and DA have potent effects throughout the body, their concentrations are tightly regulated. In the extracellular compartment, specific membrane bound transporter proteins that mediate re-uptake from the extracellular space are the most important regulators of catecholamine levels (Torres and Amara, 2007).

A range of transporter proteins expressed primarily in neuronal tissue have been characterized and include the 5-hydroxytryptamine (5-HT) transporter (Blakely *et al.*, 1991), norepinephrine transporter (NET) (Ramamoorthy *et al.*, 1993) and the dopamine transporter (Uddin *et al.*, 1991). These neuronal monoamine transporters function not only as part of the metabolizing system but perhaps more importantly as part of recycling systems to maintain the catecholamine levels in the vesicular stores (Eisenhofer, 2001).

In the extraneuronal tissue, the three known transporter proteins responsible for extraneuronal corticosterone-sensitive monoamine uptake are the organic anion transporters (OCT1, OCT2) and the extraneuronal monoamine transporter (EMT) (Gorboulev *et al.*, 1997; Grundemann *et al.*, 1998), which are mainly expressed in the intestine, liver, kidney and placenta (Kekuda *et al.*, 1998; Hayer-Zillgen *et al.*, 2002). They have a broad compound affinity, transporting all biogenic amines as well as exogenous drugs and xenobiotics (Eisenhofer, 2001). Unlike the neuronal monoamine transporters which regulate synaptic levels of monoamines, extraneuronal transporters play a

key role in clearance of monoamines from the blood stream. In general, neuronal reuptake is quantitatively more important than extraneuronal uptake for clearance of neuronally released catecholamines but the clearance of circulating catecholamines is mainly by non-neuronal mechanisms (Eisenhofer, 2001).

Neuronal metabolism of recaptured catecholamines is by oxidative deamination catalysed by monoamine oxidase (MAO) or by re-packing in vesicles in order to be available for re-release. Vesicular monoamine transporters (VMAT1 and VMAT2) mediate the accumulation of monoamines into vesicles (Peter *et al.*, 1995). In extraneuronal tissue, subsequent metabolism of catecholamines following transport uptake involves MAO and catechols-O-methyltransferase (COMT), as well as sulfation processes.

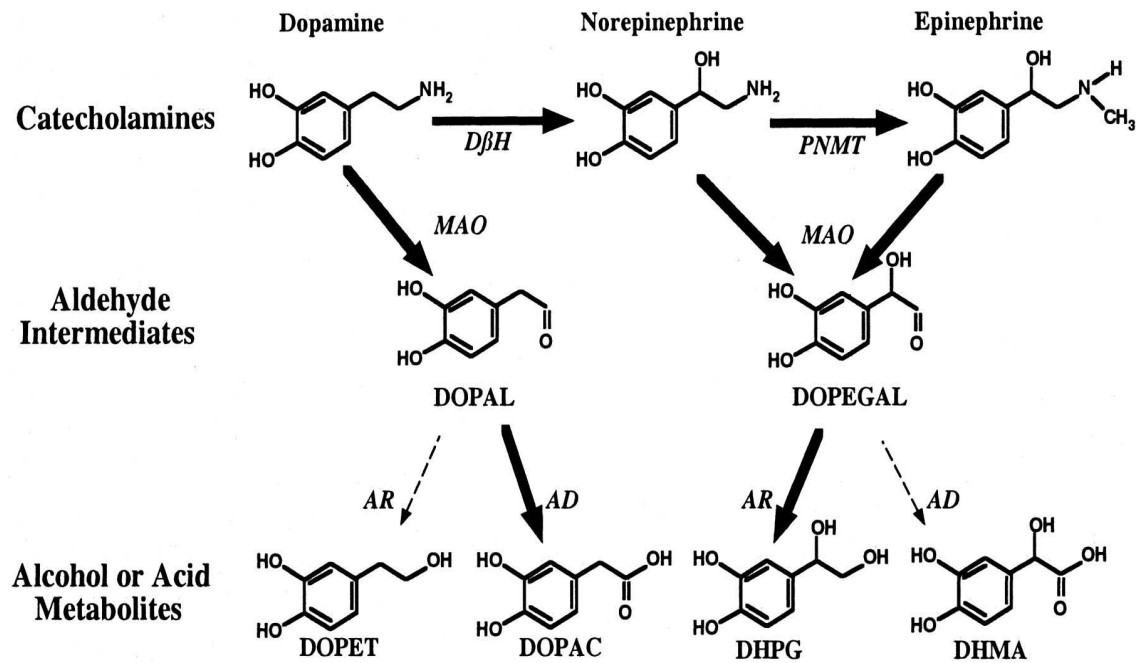
Oxidative deamination is performed by MAO, an integral protein of the outer mitochondrial membrane (Greenawalt and Schnaitman, 1970) which consists of two isoenzymes, MAO-A and MAO-B (Billett, 2004) which are encoded by different genes (Bach *et al.*, 1988; Grimsby *et al.*, 1991), have different substrate preferences (Billett, 2004), and tissue and cell distributions (Boulton and Eisenhofer, 1998). MAO-A oxidises serotonin (5-hydroxytryptamine) and NE preferentially, whereas MAO-B oxidises phenylethylamine preferentially in normal physiological conditions (Billett, 2004). In humans, MAO-A is found in the highest concentration in the liver, lung and intestine whereas MAO-B is predominant in the brain and liver

(Boulton and Eisenhofer, 1998). MAO is the only metabolising enzyme present in catecholaminergic neurons (Boulton and Eisenhofer, 1998).

Oxidative deamination of catecholamines involves a two-step reaction where MAO catalyses the first step, deaminating DA to 3,4-dihydroxyphenylacetaldehyde (DOPAL) and NE and EPI to 3,4-dihydroxyphenylglycolaldehyde (DOPEGAL) (Eisenhofer *et al.*, 2004, Figure 1.2). DOPAL and DOPEGAL are further metabolised by aldehyde dehydrogenase to DOPAC and 3,4-dihydroxymandelic acid (DHMA) respectively. Alternatively DOPAL and DOPEGAL can be metabolised by aldehyde reductase to 3,4-dihydroxyphenylethanol (DOPET) and DHPG respectively (Figure 1.2).

Figure 1.2: Pathways of oxidative deamination of catecholamines

(Adapted from Eisenhofer *et al.*, 2004 with permission).



DβH dopamine β-hydroxylase, **PNMT** phenylethanolamine-*N*-methyltransferase, **AR** aldose or aldehyde reductase, **AD** aldehyde dehydrogenase

COMT catalyses the O-methylation of the 3-hydroxyl group of most catechols in extraneuronal tissue (Goldstein *et al.*, 2003). In the presence of COMT, the substrates of L-DOPA, DA, NE and EPI are converted to 3-methoxytyrosine, 3-methoxytyramine, normetanephrine and metanephrine respectively (Eisenhofer *et al.*, 2001, Figure 1.1). COMT is expressed in high concentrations in liver, kidney, extraneuronal cells and adrenomedullary chromaffin cells (Eisenhofer *et al.*, 1998).

Sulfoconjugation represents an important protective mechanism for inactivating catecholamines, so protecting the body from the adverse effects of excess catecholamines (Boulton and Eisenhofer, 1998). Catecholamines in humans and higher primates, in contrast to other animals, circulate mainly (up to 99%) in the sulfoconjugated forms (Dousa and Tyce, 1988; Eisenhofer *et al.*, 1999). Sulfoconjugation is catalysed by members of the sulfotransferase (SULT) enzyme family, which to date comprises at least 12 isoforms in human tissues (Coughtrie, 2002). The SULT1 and SULT2 families are the largest and probably the most important for endobiotic and xenobiotic metabolism (Coughtrie, 2002). Sulfoconjugation of catecholamines is catalysed by SULT1A3 (Richard *et al.*, 2001).

SULT1A3 is widely expressed in the human fetus but the adrenal and thyroid glands have little or no enzyme activity (Stanley *et al.*, 2005). Early in fetal life, SULT1A3 is expressed at high levels in liver but this decreases significantly in the late fetal-early neonatal period (Richard *et al.*, 2001). In contrast, the enzyme SULT1A3 is essentially absent in adult human liver

(Richard *et al.*, 2001). Developing liver haematopoietic cells have substantial expression of SULT1A3 (Richard *et al.*, 2001). In human fetal brain, SULT1A3 activity is low and widespread in various brain regions (Stanley *et al.*, 2005). SULT1A3 is expressed at high levels in placenta from 13 weeks gestation with no significant developmental change to term, and in general activities are higher in the villous than membranous tissues (Stanley *et al.*, 2001).

1.4 AIM OF THESIS

At present there is little information about the normal hormonal and metabolic profiles in term and preterm infants at the time of delivery. Work that exists has focussed on single or small combinations of hormones which can create a very misleading picture. The developmental deficiencies described predominantly in preterm, but also in some term infants, in the failures of expression of the postnatal hepatic G-6-Pase system suggest that there are deficiencies in the mechanism to switch on this key enzyme of gluconeogenesis at the time of birth. The evidence for hormonal regulation of this enzyme system in animal studies, in adult humans, and in studies of cell lines make the possibility of failure of hormonal control around the time of birth an important hypothesis to test. Before this can be done, however, comprehensive studies of perinatal metabolism and its hormonal control need to be undertaken. The aim of this thesis is to describe comprehensively the hormonal and metabolic profiles of term infants delivered by elective caesarean section.

1.4.1 Objectives

1. To quantify the proportion of term infants who are hypoglycaemic at birth.
2. To describe the hormonal and metabolic responses in the regulation of blood glucose levels in infants delivered by elective caesarean section.
3. To describe the relationship between maternal and infant glucose status at delivery.

CHAPTER 2

PILOT STUDY 1: GLUCOSE AND LACTATE LEVELS IN CORD BLOOD SAMPLES ROUTINELY COLLECTED AT DELIVERY FOR ESTIMATION OF BLOOD GAS AND ACID-BASE STATUS IN TERM INFANTS

2.1 INTRODUCTION

The aim of the first pilot study was to determine the incidence of hypoglycaemia in infants at delivery and to identify any antenatal or intrapartum factors associated with hypoglycaemia. Since 1994, sampling of umbilical arterial and venous cord blood for blood gas and acid-base status has been routine practice for all deliveries in Ninewells Hospital and Medical School, Dundee. It was decided to use this established practice to determine the incidence of hypoglycaemia in term infants and to identify any antenatal or intrapartum factors which might be associated with hypoglycaemia. As described previously (see Chapter 1 pages 4-10) the definition of what constitutes hypoglycaemic blood glucose levels in infants remains contentious, but as evidence to support a level higher than 2.6 mmol/l is lacking, hypoglycaemia was pre-defined as a blood glucose concentration less than 2.6 mmol/l.

2.2 METHODS

2.2.1 Mothers and infants

Three hundred and ninety nine infants between 37 and 42 weeks gestation who were delivered in Ninewells Hospital and Medical School, Dundee were recruited from April to July 2003. The study population of Dundee is stable with a reported 92.45% of the population staying in the same area according to the 2001 census (General Register Office for Scotland, 2003). Dundee has a very small ethnic minority population; the majority of the population is white (96.34%) and the remainder: Indian, Pakistani and other South Asian, Chinese and others (General Register Office for Scotland, 2003). About 80% of pregnant women living in Tayside deliver in Ninewells Hospital, Dundee and this study aimed to recruit a consecutive sample of eligible pregnant women. Exclusion criteria were known Hepatitis B, Hepatitis C or HIV positivity, or if the mother was unable to provide informed consent.

Mothers were given the Study Information Sheets by a midwife (see Appendix pages 144-145) when they were admitted to the labour ward. All mothers had a period of at least 24 hours before a decision regarding consent was sought; written informed consent was obtained for all participants. The study was approved by the Tayside Committee on Medical Research Ethics (Ref: 033/04). If mothers agreed to participate, they were asked to allow an extra sample of blood to be taken at the time of the routine blood gas and acid-base status measurements of their infant and to allow data to be abstracted from their medical records. If it was not possible to obtain consent before delivery, the extra blood sample could be obtained

after delivery but before it was analysed for lactate and glucose levels. Samples would be discarded if the mothers declined to participate.

2.2.2 Blood sample collection

The midwife who primarily looked after the mother in labour collected cord arterial and venous blood for measurement of blood gas levels and acid-base status. Blood gas levels and acid-base status were analysed in an AVL OMNI Modular System blood gas analyser (Roche Diagnostics GmbH, Graz, Austria) which is located in the labour ward and serviced by the Department of Biochemical Medicine, Ninewells Hospital and Medical School as part of the UK national accreditation scheme.

At the same time of this routine clinical practice, an additional (approximately) five ml of blood was taken by the midwife from the umbilical vein using a 19 gauge needle (Hospira, Lake Forest, USA) for glucose and lactate measurements. The method of sample collection was not standardised on the ward and could be from either a single or double clamped cord. The blood sample was collected into a fluoride-oxalate tube and stored in a fridge for a maximum of 12 hours. The blood sample was sent for analysis of glucose and lactate levels in the Department of Biochemical Medicine, Ninewells Hospital and Medical School.

2.2.3 Data collection

There is limited information about the antenatal, intrapartum or postpartum factors which affect fetal glucose homeostasis. Therefore, a range of

epidemiological data was abstracted from the case notes about maternal health, labour, delivery and infant characteristics. Extensive antenatal data of the women were collected which included age and smoking history; booking height and weight, and delivery weight; presence of disease including: antepartum haemorrhage, asthma, epilepsy, thyroid disease, diabetes, hypertension, treated genito-urinary and vaginal infection (in the week before delivery); antenatal medications such as tocolytic, anticonvulsant, antihypertensive and corticosteroid usage. Body mass index (BMI) was calculated for each woman using booking weight and height. Extensive labour details were collected including multiple birth, time of onset of labour, use of oxytocin, mode of delivery, drugs and fluid received during labour, analgesia, duration of labour; Apgar scores at one and five minutes, mode of resuscitation (facial oxygen, intermittent positive pressure ventilation by mask or tube and drugs) and placental weight. Infant characteristics collected included occipital frontal circumference (OFC), length, gender, birth weight and birth weight ratio. Birth-weight ratios were calculated for each infant using reference values (denominator) obtained from the Scottish Morbidity Record SMR2 (supplied by the Information and Statistics Division of the Common Services Agency, Edinburgh). The denominator was the mean weight for each week of gestation for all infants born in Scotland from 1987 to 1998 (See Appendix X for a copy of the Data Abstraction Form).

2.2.4 Data handling and Statistical analyses

All data were entered into Epi Info™ version 3.4.3 by an experienced data manager, doubled checked, and verified. Any discrepancies were identified,

checked and rectified as appropriate. Data were exported from Epi Info™ to SPSS version 14 statistical software for analysis. The association of maternal and infant antenatal, intrapartum and postpartum factors with infant hypoglycaemia (glucose levels less than 2.6 mmol/l) in venous cord blood samples were determined using the Student's two tailed *t*-test for continuous variables and the Fisher exact test for categorical variables. Means and standard deviations (SD) of glucose, lactate, pH, pO₂, pCO₂, bicarbonate (CHCO₃) and base excess (BE) levels were calculated for each mode of delivery (spontaneous vaginal delivery, vaginal breech, operative vaginal delivery, emergency caesarean section and elective caesarean section)

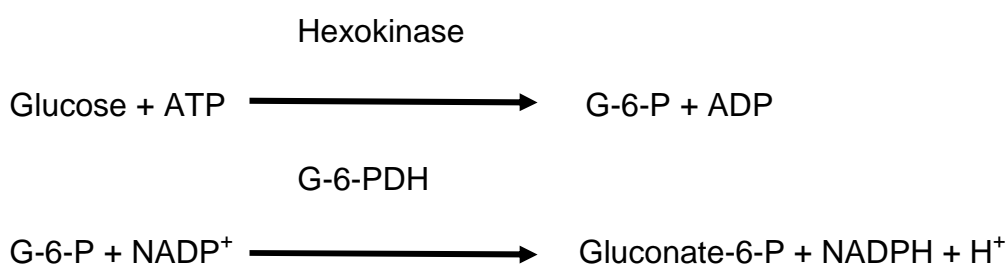
Further detailed analysis was carried out using univariate general linear modelling; the dependent factors were cord blood glucose, lactate, pH, pO₂, pCO₂, CHCO₃ and BE levels and the independent factors were type of delivery with spontaneous vaginal delivery group as the referent category.

2.2.5 Biochemical Analysis

2.2.5.1 Glucose (mmol/l)

Plasma glucose was measured using a Roche Gluco-quant kit (Roche Diagnostics GmbH, Mannheim, Germany) on a Roche Modular Serum Work Area Analyser (Roche Diagnostics GmbH, Mannheim, Germany) in the Department of Biochemical Medicine, Ninewells Hospital and Medical School, Dundee. The venous cord blood sample was collected into a fluoride-oxalate tube and stored in a fridge until onward transport to the Department of Biochemical Medicine, Ninewells Hospital and Medical

School, Dundee for analysis. The assay uses hexokinase (yeast) to catalyze the phosphorylation of glucose to G-6-P by adenosine triphosphate (ATP), which is converted to adenosine diphosphate (ADP). Glucose-6-phosphate dehydrogenase (G-6-PDH) then oxidises G-6-P in the presence of nicotinamide adenine dinucleotide (NADP) to gluconate-6-phosphate (gluconate-6-P). The reaction is specific for glucose and no other carbohydrate is oxidised. The rate of reduced nicotinamide adenine dinucleotide phosphate (NADPH) formation during the reaction is directly proportional to the glucose concentration and can be measured photometrically at 340nm.

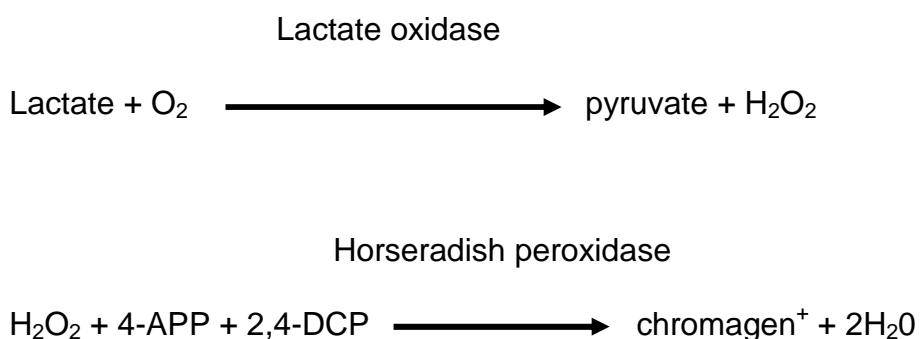


The assay measures glucose concentrations between 0.11 and 41.6 mmol/l and has an inter-assay coefficient of variation of 1.33%.

2.2.5.2 Lactate (mmol/l)

Plasma lactate was also measured based on an enzymatic method using a Cobas[®] Lactate kit (Roche Diagnostics GmbH, Mannheim, Germany) on a Roche Modular Serum Work Area Analyser (Roche Diagnostics GmbH, Mannheim, Germany), in the Department of Biochemical Medicine, Ninewells Hospital and Medical School, Dundee. L-lactate is oxidised to pyruvate by

the specific enzyme lactate oxidase with the production of stoichiometric amounts of hydrogen peroxide. Horseradish peroxidase is used to generate a coloured dye using the hydrogen peroxide generated in the first reaction. 4-aminophenazone (4-APP) as a colour coupler with sulphonated 2,4-dichlorophenol (2,4-DCP) is used to determine the amount of hydrogen peroxide produced. The intensity of the colour formed is proportional to the L-lactate concentration and is measured as an endpoint reaction at 658 nm.



The venous cord blood was collected into a fluoride-oxalate tube. The assay measures lactate concentrations between 0.2 and 15.5 mmol/l and has an inter-assay coefficient of variation of 1.7%.

2.2.5.3 Blood gas and acid-base profiles

Blood gas and acid-base analysis (pH, pO₂, pCO₂, CHCO₃, and BE) were performed on an AVL OMNI Modular System blood gas analyzer (Roche Diagnostics GmbH, Mannheim, Germany). The OMNI uses a new generation of sensors and a patented liquid calibration system for blood gases, acid-base status and electrolyte estimations. The advantage of these new sensors is that they use generally proven principles and technologies

but implement these principles in a way that the sample volume needed is exceedingly low. After each procedure, the analyser undergoes an automatic self cleaning process. Three levels of auto-calibration takes place every hour, 12 hours and 24 hours. Staff from the Department of Biochemical Medicine, Ninewells Hospital and Medical School carry out quality control checks daily and preventative maintenance services twice weekly. The blood gas analyser is also monitored in an external UK quality assurance scheme. The inter-assay coefficient of variation for pH, pO₂ and pCO₂ were 0.20%, 9.84% and 4.24% respectively.

2.3 RESULTS

Three hundred and ninety nine term infants were recruited; 11.3% (45) were hypoglycaemic at delivery with a cord venous blood glucose levels less than 2.6 mmol/l. Only one maternal antenatal factor distinguished infants who were hypoglycaemic from normoglycaemic; mothers of hypoglycaemic infants were slightly older than mothers whose infants were normoglycaemic ($p=0.024$, Table 2.1).

During the intrapartum period, more events distinguished hypoglycaemic from normoglycaemic infants. Elective caesarean section increased the likelihood of being hypoglycaemic almost sixfold (OR 5.59, $p<0.001$). The use of epidural or spinal analgesia in the women during labour and delivery doubled the likelihood of the infant being hypoglycaemic (OR 2.03, $p=0.039$). In contrast, opiate use in labour and delivery appeared to have a 'protective' effect with an odds ratio of 0.27 ($p=0.001$) in the hypoglycaemic group

compared to the normoglycaemic group (Table 2.2). Normoglycaemic infants experienced a significantly longer first ($p=0.001$) and second ($p=0.005$) stages of labour compared to hypoglycaemic infants (Table 2.2).

Seven postpartum factors distinguished hypoglycaemic from normoglycaemic infants (Table 2.3). Multiple birth was associated with an increased likelihood of hypoglycaemia in the infants (OR 6.3, $p=0.002$, Table 2.3). Hypoglycaemic infants had significantly lower umbilical cord venous pO_2 ($p=0.004$), but higher umbilical cord arterial pH ($p=0.005$), umbilical cord arterial and venous BE ($p<0.001$, $p=0.003$ respectively), umbilical cord arterial and umbilical cord venous $CHCO_3$ ($p=0.001$, $p=0.004$ respectively) compared to the normoglycaemic infants (Table 2.3). The mean birth weight was also significantly lower in the hypoglycaemic group compared to the normoglycaemic group (3365g v 3526g, $p=0.224$, Table 2.3).

Cord glucose, lactate, pH, pO_2 , pCO_2 , $CHCO_3$ and BE levels varied significantly according to the type of delivery (Table 2.4). Infants delivered by elective caesarean section had the lowest mean levels of glucose (2.73 mmol/l) and lactate (3.37 mmol/l) amongst the five groups of delivery (Table 3.4). This group of infants also had the highest cord arterial pH (7.28) and cord arterial BE (-3.43 mmol/l) levels (Table 2.4). By contrast infants delivered by operative vaginal delivery had the highest mean levels of glucose (4.2 mmol/l) and lactate (6.08 mmol/l) (Table 2.4). Compared to spontaneous vaginal deliveries, infants delivered by elective caesarean section had significantly lower mean levels of glucose (-1.26 mmol/l), lactate

(-1.97 mmol/l) and cord arterial pO_2 (-0.57 kPa); and significantly higher levels of cord arterial pCO_2 (+0.59 kPa), cord venous pCO_2 (+0.55 kPa), cord arterial BE (-2.51 mmol/l), cord venous BE (-1.54 mmol/l) cord arterial $cHCO_3$ (+2.55 mmol/l) and cord venous $cHCO_3$ (+1.93 mmol/l) (Table 2.4).

Table 2.1: Association of maternal antenatal factors with infant hypoglycaemia (glucose levels less than 2.6 mmol/l) in venous cord blood samples

	Hypoglycaemic infants Mean \pm SD (n) or N %	Normoglycaemic infants Mean \pm SD (n) or N %	Test statistic t-statistic (t) or Odds ratio (95% CI)	p value[#]
Maternal age, years	30.93 \pm 6.12 (45)	28.69 \pm 6.67 (353)	t=2.29	0.024*
Maternal BMI	26.20 \pm 4.54 (42)	25.44 \pm 5.16 (323)	t=1.00	0.322
Maternal smokers	13/104, 12.5%	91/104, 87.5%	OR=1.16 (0.582, 2.299)	0.733
Antenatal events:				
Antepartum haemorrhage	2/32, 6.3%	30/32, 93.7%	OR=0.52 (0.120, 2.259)	0.586
Asthma	3/40, 7.5%	37/40, 92.5%	OR=0.61 (0.180, 2.066)	0.620
Epilepsy	2/8, 25.0%	6/8, 75.0%	OR=2.69 (0.526, 13.750)	0.451
Thyroid disease	2/10, 20.0%	8/10, 80.0%	OR=2.02 (0.415, 9.809)	0.630
Diabetes	0/4, 0%	4/4, 100%	-	-
Hyperemesis gravidarum	2/13, 4.4%	11/13, 84.6%	OR=1.43 (0.307, 6.684)	0.896
Tractocile treatment	0/1, 0%	1/1, 100%	-	-
Nifedipine treatment	2/4, 50.0%	2/4, 50.0%	OR=8.16 (1.121, 59.430)	0.130
Betamethasone injection	4/16, 25.0%	12/16, 75.0%	OR=2.77 (0.855, 8.995)	0.188
Hypertension	4/24, 16.7%	20/24, 83.3%	OR=1.63 (0.531, 5.000)	0.560
Treated UTI	0/3, 0%	3/3, 100%	-	-
Treated vaginal discharge	1/3, 33.3%	2/3, 66.7%	OR=3.99 (0.354, 44.890)	0.606

BMI body mass index, **OR** odd ratio, **UTI** urinary tract infection

[#] Student *t*-test or Fisher-exact test

Significant * *p* < 0.05

Table 2.2: Association of maternal intrapartum factors with hypoglycaemia (glucose levels less than 2.6 mmol/l) in venous cord blood samples

	Hypoglycaemic infants Mean \pm SD (n) or N %	Normoglycaemic infants Mean \pm SD (n) or N %	Test statistic t-statistic (t) or Odds ratio (95% CI)	p value[#]
Labour onset - spontaneous	19/243, 7.8%	224/243, 92.2%	OR=0.67 (0.326, 1.440)	0.318
Labour augmented	7/94, 7.4%	87/94, 92.6%	OR=0.87 (0.371, 2.080)	0.968
Type of delivery				
Spontaneous vaginal delivery	24/255, 9.4%	231/255, 90.6%	OR=0.61 (0.326, 1.137)	0.163
Vaginal breech	1/3, 33.3%	2/3, 66.7%	OR=4.00 (0.355, 45.021)	0.605
Instrumental cephalic vaginal	3/65, 4.6%	62/65, 95.4%	OR=0.34 (0.101, 1.120)	0.083
Emergency caesarean section	4/39, 10.3%	35/39, 89.7%	OR=0.89 (0.301, 2.630)	1.080
Elective caesarean section	13/37, 35.1%	24/37, 64.9%	OR=5.59 (2.596, 12.020)	<0.001***
Type of analgesia received				
None received	2/24, 8.3%	22/24, 91.7%	OR=0.70 (0.159, 3.080)	0.950
Entonox	28/292, 9.6%	264/292, 90.4%	OR=0.55 (0.290, 1.062)	0.112
General anaesthesia	0/3, 0%	3/3, 100%	-	-
Epidural/spinal	27/178, 15.2%	150/178, 84.3%	OR=2.03 (1.078, 3.821)	0.039*
Opiate	7/149, 4.7%	142/149, 95.3%	OR=0.27 (0.119, 0.630)	0.001**
Duration of first stage mins	256.93 \pm 279.57 (45)	417.31 \pm 386.99 (354)	t=-3.45	0.001**
Duration of second stage mins	28.89 \pm 55.65 (45)	54.68 \pm 62.91 (352)	t=-2.88	0.005**
Duration of membrane ruptured mins	525.44 \pm 966.43 (45)	463.46 \pm 634.15 (352)	t=0.42	0.677

OR odd ratio

Student t-test or Fisher-exact test

Significant * p< 0.05, ** p< 0.01, *** p< 0.001

Table 2.3: Association of infant factors with hypoglycaemia (glucose levels less than 2.6 mmol/l) in venous cord blood samples

	Hypoglycaemic infants Mean \pm SD (n) or N %	Normoglycaemic infants Mean \pm SD (n) or N %	Test statistic t-statistic (t) or Odds ratio (95% CI)	p value[#]
Male infant	19/191, 10.0%	172/191, 90.0%	OR=0.77 (0.408, 1.432)	0.497
Multiple births	7/17, 41.2%	10/17, 58.8%	OR=6.30 (2.266, 17.510)	0.002**
Apgar score < 3 at 1 min	0/45, 0%	0/352, 0%	-	-
Apgar score < 7 at 5 min	0/45, 0%	0/353, 0%	-	-
Cord blood arterial pH	7.28 \pm 0.06	7.25 \pm 0.01 (336)	t=2.05	0.005**
Cord blood venous pH	7.31 \pm 0.06	7.31 \pm 0.08 (262)	t=0.51	0.610
Cord blood arterial BE	-4.21 \pm 2.21 (43)	-6.01 \pm 2.98 (336)	t=4.81	<0.001***
Cord blood venous BE	-3.95 \pm 1.84 (27)	-5.17 \pm 2.50 (262)	t=3.16	0.003**
Cord blood arterial pO ₂ kPa	3.03 \pm 1.40 (42)	2.95 \pm 1.13 (331)	t=0.36	0.723
Cord blood venous pO ₂ kPa	3.31 \pm 0.94 (27)	4.10 \pm 3.15 (258)	t=-2.96	0.004**
Cord blood arterial pCO ₂ kPa	6.67 \pm 1.18 (43)	6.86 \pm 1.70 (336)	t=-0.97	0.333
Cord blood venous pCO ₂ kPa	5.99 \pm 1.26 (27)	5.75 \pm 1.21 (262)	t=0.93	0.359
Cord blood arterial CHCO ₃ mmol/l	22.89 \pm 2.19 (43)	21.62 \pm 2.65 (335)	t=3.50	0.001**
Cord blood venous CHCO ₃ mmol/l	22.18 \pm 2.17 (27)	20.84 \pm 2.17 (262)	t=3.07	0.004**
Neonatal resuscitation	4/29, 13.8%	25/29, 86.2%	OR=1.28 (0.423, 3.850)	0.844
Infant birth weight grams	3365.44 \pm 669.25 (45)	3526.06 \pm 1613.31 (354)	t=-1.22	0.224
Infant birth weight ratio	1.01 \pm 0.15 (44)	1.00 \pm 0.13 (352)	t=0.41	0.686
Infant OFC cm	34.52 \pm 1.53 (45)	34.84 \pm 1.62 (354)	t=-1.30	0.198
Infant birth length cm	49.06 \pm 2.75 (45)	49.48 \pm 2.36 (354)	t=-0.97	0.336

BE base excess, **CHCO₃** bicarbonate, **OFC** occipital-frontal head circumference, **OR** odd ratio

[#] Student t-test or Fisher-exact test

Significant ** p < 0.01, *** p<0.001

Table 2.4: The relationship of mode of delivery and levels of cord blood glucose, lactate, pH, pO₂, pCO₂, bicarbonate and base excess

	Spontaneous vaginal delivery Mean \pm SD (n)	Vaginal breech Mean \pm SD (n)	Operative Vaginal delivery Mean \pm SD (n)	Emergency caesarean section Mean \pm SD (n)	Elective caesarean section Mean \pm SD (n)
Glucose mmol/l	3.99 \pm 1.17 (254)	3.83 \pm 2.14 (3)	4.2 \pm 1.04 (65)	3.64 \pm 1.04 (39)	2.73 \pm 0.60 (36)***
Lactate mmol/l	5.34 \pm 1.75 (253)	5.60 \pm 0.85 (3)	6.08 \pm 2.49 (65)**	5.06 \pm 2.76 (39)	3.37 \pm 1.32 (35)***
Cord blood arterial pH	7.26 \pm 0.10 (239)	7.16 \pm 0.14 (3)	7.23 \pm 0.09 (65)*	7.23 \pm 0.11 (36)	7.28 \pm 0.09 (36)
Cord blood venous pH	7.32 \pm 0.07 (180)	7.27 \pm 0.09 (2)	7.27 \pm 0.08 (47)***	7.27 \pm 0.10 (32)	7.32 \pm 0.06 (28)
Cord blood arterial BE mmol/l	-5.94 \pm 2.76 (239)	-5.73 \pm 1.66 (3)	-6.70 \pm 2.86 (65)	-5.7 \pm 3.89 (36)	-3.43 \pm 2.20 (36)***
Cord blood venous BE mmol/l	-4.91 \pm 2.11 (180)	-5.8 \pm 0.14 (2)	-6.19 \pm 2.73 (47)***	-5.6 \pm 3.45 (32)	-3.37 \pm 1.92 (28)**
Cord blood arterial pO ₂ kPa	3.14 \pm 1.14 (235)	2.69 \pm 1.09 (3)	2.94 \pm 1.21 (64)	2.19 \pm 0.84 (35)***	2.57 \pm 1.18 (36)**
Cord blood venous pO ₂ kPa	4.40 \pm 3.69 (178)	4.58 \pm 2.16 (2)	3.56 \pm 0.99 (46)	2.89 \pm 0.93 (31)*	3.60 \pm 1.00 (28)
Cord blood arterial pCO ₂ kPa	6.63 \pm 1.64 (239)**	9.62 \pm 4.12 (3)**	7.07 \pm 1.69 (65)	7.23 \pm 1.53 (36)*	7.22 \pm 1.08 (36)*
Cord blood venous pCO ₂ kPa	5.50 \pm 1.07 (180)	6.42 \pm 2.11 (2)	6.13 \pm 1.28 (47)	6.49 \pm 1.58 (32)***	6.05 \pm 0.95 (28)*
Cord blood arterial cHCO ₃ mmol/l	21.47 \pm 2.51 (238)	24.33 \pm 3.50 (3)	21.16 \pm 2.65 (65)	22.31 \pm 2.39 (36)	24.02 \pm 2.29 (36)***
Cord blood venous cHCO ₃ mmol/l	20.70 \pm 2.08 (180)	21.2 \pm 2.69 (2)	20.61 \pm 2.29 (47)	21.47 \pm 2.15 (32)	22.63 \pm 2.19 (28)***

Spontaneous vaginal delivery is the reference category

Significant * p<0.05, ** p<0.01, *** p<0.001

2.4 COMMENTARY

Compared to other modes of delivery, infants delivered by elective caesarean section had the highest pH and BE but lowest glucose and lactate levels (Table 2.4). Similar results for pH and BE levels in infants delivered by elective caesarean section have been reported in other studies (Suidan *et al.*, 1984; Nodwell *et al.*, 2005). It has been suggested that the umbilical cord gases of infants delivered by caesarean section, without labour, results in values more closely approximating adult blood gas levels and acid-base status (Johnson *et al.*, 1990). In terms of fetal metabolic and hormonal responses to mode of delivery, infants delivered by elective caesarean section have the lowest lactate (Suidan *et al.*, 1984; Shirey *et al.*, 1996), cortisol (Mears *et al.*, 2004; Vogl *et al.*, 2006), EPI and NE levels (Irestedt *et al.*, 1982; Wang *et al.*, 1999) compared to other mode of deliveries. It could be argued that infants delivered by elective caesarean section were in the most 'stress-free' environment compared to other modes of delivery. Following this argument, it appears contradictory that the incidence of hypoglycaemia was higher in infants delivered by elective caesarean section than in those delivered by emergency caesarean section or spontaneous vaginal delivery (Table 2.2).

These contradictory results raised the question of the validity of the results, with the possibility that the glucose levels might have changed significantly with time. There is clear evidence from the literature that cord blood gas levels and acid-base status change with time post-delivery (Sykes and Molloy, 1984; Duerbeck

et al., 1992; Armstrong and Stenson, 2006; Lynn and Beeby, 2007); but there is a paucity of evidence on levels of other blood sample constituents – such as lactate and glucose. Currently, there is limited evidence about the changes in cord blood lactate levels with time post-delivery (Armstrong and Stenson, 2006), and there are no comparable studies on blood glucose, which is the likely major precursor for cord blood lactate generation subsequent to delivery.

This rationale led to a further pilot study (pilot study 2) which was designed to investigate the changes with time after delivery in umbilical cord blood levels of glucose, lactate, pH, pO₂, pCO₂, cHCO₃ and BE.

CHAPTER 3

PILOT STUDY 2: MEASURING CHANGES IN VENOUS CORD BLOOD GLUCOSE, LACTATE, pH, pO₂, pCO₂, BICARBONATE AND BASE EXCESS VALUES WITH TIME FOLLOWING DELIVERY

3.1 INTRODUCTION

The purpose of this study was to measure the change in venous cord blood glucose, lactate, pH, pO₂, pCO₂, cHCO₃ and BE values post-delivery. Previous work in this area has used fairly wide time intervals and ranged from 20 to 30 minutes (e.g. Armstrong and Stenson, 2006; Lynn & Beeby, 2007) and it is possible that the time taken for levels to reduce significantly was obscured. To provide a finer detail of information, we decided to monitor the changes in levels at eight points during the first 62 minutes post-delivery.

3.2 METHODS

3.2.1 Mothers and infants

Twenty-two women who had an elective caesarean section for a singleton pregnancy at term (37 to 42 weeks) in Ninewells Hospital and Medical School, Dundee were recruited over a five month period from May to September 2005. All women had an uneventful antenatal period. Gestational age was calculated using the dating ultrasound scan taken in the first trimester. Exclusion criteria were known Hepatitis B, Hepatitis C or HIV positivity, placenta praevia, or if the

mother was unable to provide informed consent. Women who had placenta praevia were excluded in this study because of the difficulty in obtaining a sufficient volume of blood for analysis. All women fasted for at least six hours prior to their caesarean section.

Eligible women were identified by looking through the Labour Suite work diary. Once identified, a letter inviting the woman to join the study was sent out at least one week before their elective caesarean section date. Recruitment and consent were obtained in the morning of the day the woman was admitted for delivery. All women had a period of at least 24 hours before making a decision about participation in the study. In all cases written informed consent was obtained. The study was approved by the Tayside Committee on Medical Research Ethics (Ref: 033/04).

3.2.2 Blood sample collection

Cord blood samples for measurement of glucose, lactate and blood gas levels, and acid-base status were obtained by me from the umbilical vein at the placental cord insertion i.e. single clamped samples using a 19 gauge Butterfly needle (Hospira, Lake Forest, USA) at 2, 7, 12, 22, 32, 42, 52, and 62 minutes after delivery of infants. Post-delivery placentas were placed in an open container, at room temperature, during the blood sampling period. Venous blood samples were collected into one ml heparinised plastic syringes (BD Diagnostics, Plymouth, UK) for blood gas estimations and into fluoride–oxalate

tubes for glucose and lactate determinations. Blood gases were analysed immediately on sampling in an AVL OMNI Modular System blood gas analyser (Roche Diagnostics GmbH, Graz, Austria) in the labour ward whereas blood samples in fluoride-oxalate tubes for glucose and lactate levels were batched and sent to the Department of Biochemical Medicine, Ninewells Hospital and Medical School, Dundee for analysis at the end of an hour's study.

3.2.3 Statistical analyses

Data were entered into a Microsoft Excel XP spreadsheet, checked and verified before use. The analysis was carried out using Microsoft Excel XP. Means and SD values were calculated for venous cord blood glucose, lactate, pH, pO₂, pCO₂, CHCO₃ and BE values at 2, 7, 12, 22, 32, 42, 52, and 62 minutes after delivery. The relative temporal changes in venous cord blood glucose, lactate, pH, pO₂, pCO₂, CHCO₃ and BE values following delivery compared to baseline levels were determined using the Student's two tailed *t*-test. Baseline levels was the first cord blood sample which was taken at two minutes post-delivery of the infant and which allowed time for delivery of the placenta.

3.2.4 Biochemical Analysis

3.2.4.1 Glucose

The glucose analysis was identical to that of the first pilot study (see page 40-41 for details).

3.2.4.2 Lactate

The lactate analysis was identical to that of the first pilot study (see pages 41-42 for details).

3.2.4.3 Blood gas and acid-base profiles

The analysis of blood gas and acid-base status was identical to that of the first pilot study (see pages 42-43 for details).

3.3 RESULTS

During the 62 minutes after delivery, there were considerable changes in venous cord blood glucose, lactate, pH, pO₂, pCO₂, cHCO₃ and BE values in term infants delivered by elective caesarean section (Table 3.1, Figure 3.1). Glucose levels decreased over time and were significantly lower at 12 minutes compared to the baseline levels at two minutes post-delivery of infants ($p<0.01$). There was a gradual increase in lactate levels which were significantly different from the baseline levels by seven minutes post-delivery of infants ($p<0.001$). Cord venous pH ($p<0.01$) and pO₂ ($p<0.01$) values fell steadily over time and were significantly lower at seven minutes post-delivery of infants compared to the baseline values at two minutes. The base excess decreased (more negative) significantly from seven minutes post-delivery of infants compared to the baseline values ($p<0.001$). From 12 minutes post-delivery of infants, there were increments in pCO₂ tensions compared to baseline levels ($p<0.01$).

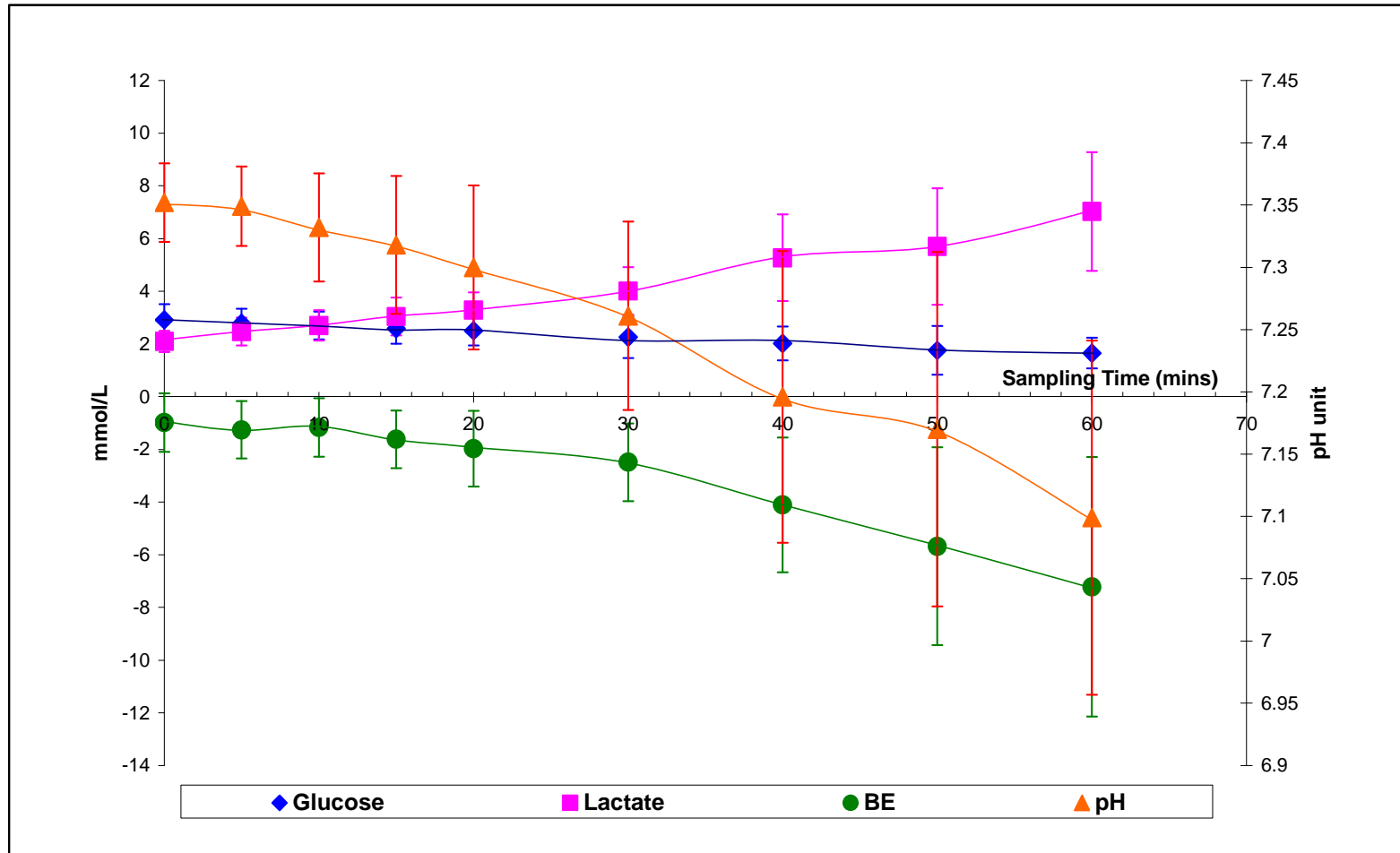
Table 3.1: Venous cord blood glucose, lactate, pH, pO₂, pCO₂, cHCO₃ and BE values with time following delivery of infants by elective caesarean section

Time (mins)	Glucose (mmol/l) Mean±SD (n)	Lactate (mmol/l) Mean±SD (n)	pH Mean±SD (n)	pO ₂ (kPa) Mean±SD (n)	pCO ₂ (kPa) Mean±SD (n)	cHCO ₃ (mmol/l) Mean±SD (n)	BE (mmol/l) Mean±SD (n)
2	3.01±0.58 (21)	2.09±0.40 (19)	7.35±0.03 (22)	2.61±0.71 (22)	6.17±0.66 (22)	24.97±1.49 (22)	-0.98±1.11 (22)
7	2.88±0.55 (21)	2.46±0.53 (19)***	7.34±0.03 (22)**	2.54±0.71 (22)**	6.32±0.76 (22)	24.93±1.62 (22)	-1.26±1.09 (22)***
12	2.79±0.53 (21)**	2.70±0.58 (19)***	7.33±0.04 (22)**	2.33±0.68 (22)***	6.55±1.03 (22)**	25.23±1.78 (22)	-1.17±1.11 (22)
17	2.69±0.57 (21)***	3.04±0.72 (19)***	7.32±0.06 (22)**	2.22±0.72 (22)***	6.77±1.27 (22)**	25.14±1.71 (22)	-1.62±1.10 (22)**
22	2.60±0.57 (20)***	3.27±0.67 (18)***	7.30±0.07 (21)***	2.17±0.70 (21)***	7.13±1.45 (21)**	25.23±1.43 (22)	-1.98±1.43 (21)***
32	2.42±0.80 (19)***	3.98±0.90 (17)***	7.26±0.08 (20)***	2.06±0.74 (20)***	7.60±2.87(20)*	25.71±1.86 (22)	-2.49±1.48 (20)***
42	2.14±0.64 (17)***	5.20±1.64 (15)***	7.20±0.12 (18)***	1.94±0.75 (18)***	9.37±2.88 (18)***	25.54±1.39 (18)	-4.11±2.55 (18)***
52	2.04±0.93 (8)***	5.55±2.21 (6)**	7.17±0.14 (8)**	2.19±0.83 (8)***	9.49±3.07 (8)*	24.33±1.35 (8)	-5.68±3.76 (8)**
62	1.78±0.59 (16)***	6.95±2.25 (14)***	7.10±0.17 (16)***	1.91±0.70 (16)***	11.40±4.18 (16)***	24.45±2.05(16)	-7.22±4.92 (16)***

cHCO₃ bicarbonate, BE base excess

* p< 0.05, **p<0.01, ***p<0.001

Figure 3.1: Venous cord blood glucose, lactate, pH, pO₂, pCO₂, CHCO_3 and BE values with time following delivery of infants by elective caesarean section



3.4 COMMENTARY

The importance of measuring umbilical cord blood gas to quantify the acid-base status of infants at birth was first recognised by James and colleagues in 1958 (James *et al.*, 1958). It has since been widely accepted that measurement of umbilical cord gas and acid-base status gives an important measure of the infant's condition at birth. Many studies have investigated the influence of sampling site and time interval after delivery on umbilical cord blood gas levels and acid-base status (Sykes and Molloy, 1984; Duerbeck *et al.*, 1992; Armstrong and Stenson, 2006; Lynn and Beeby, 2007), but there is a paucity of evidence on how soon these occur post-delivery, and of the modifications that might occur in levels of other blood sample constituents – such as lactate and glucose. This pilot study is the first to report data on the change of glucose levels with time in umbilical cord blood post-delivery.

During the 62 minutes after delivery, there were significant changes in venous cord blood glucose, lactate, pH, pO₂, pCO₂, cHCO₃ and BE values with time. For instance depending on the time lapse in analysing venous cord glucose levels, an infant might be categorised as normoglycaemic if sampled up to 21 minutes post-delivery, but had the sample analysis been delayed to 22 or more minutes, such an infant could be categorised as hypoglycaemic and in need of clinical management (Hume, 1998). This novel observation of a temporal change in blood glucose levels post-delivery is perhaps not surprising given the extensive previous literature on increments of cord blood lactate levels *in-situ* in the cord and placenta post-

delivery (Armstrong and Stenson, 2006; Lynn and Beeby, 2007). The majority of circulating blood cells in the fetus at term are non-nucleated red blood cells without mitochondria and hence no capacity for aerobic glycolysis. There may be minor contributions to aerobic glycolysis in the placenta and cord from nucleated red blood cells, white blood cells and platelets, as well as from endothelial lining cells, but the majority of blood glucose consumed will be metabolised to lactic acid and not carbon dioxide. This phenomenon of glucose consumption and lactate production in blood samples is well known and is the basis for the addition of fluoride-oxalate to inhibit glycolysis in blood samples in-transit to the laboratory. The significant changes in reducing oxygen and increasing carbon dioxide levels suggest aerobic glycolysis but as the majority of circulating blood cells in the fetus at term are non-nucleated red blood cells without mitochondria there is therefore no capacity for aerobic glycolysis. The likely explanation is due to the artefact of the model as successive blood sampling draws blood from the placenta. This is further supported by the result findings of Owen and colleagues who have shown no significant changes in pO_2 and pCO_2 levels from a double clamped umbilical vein over 30 minutes (Owen *et al.*, 1995).

There is limited evidence about the changes in cord blood lactate levels with time post-delivery. In a recent study, Armstrong and Stenson showed that cord arterial and venous lactate levels from unclamped arteries (effectively single clamped) were significantly higher by 20 minutes post-delivery compared to baseline, but it is important to note that in their study the blood samples were taken at three 20 minute intervals (up to 60 minutes) post-

delivery (Armstrong and Stenson, 2006). The results of this current pilot study showed that cord venous blood lactate levels had changed significantly as early as seven minutes post-delivery; that the lactate level gradually increases with time, implies continuous anaerobic glycolysis in the blood cells trapped in the placenta. It has also been shown that the lactate level increased significantly and within five minutes in a syringe blood sample stored at room temperature (Sinn *et al.*, 2001). In recent years, lactate measurements in cord or fetal scalp samples have been evaluated as a potential tool in the assessment of fetal status at birth as well as a measure of the degree of intrapartum hypoxia (Wiberg-Itzel *et al.*, 2008) as blood lactate analysing devices only need five μ l of blood as opposed to 30 μ l to 50 μ l for blood gas analysis. Furthermore, fetal scalp blood gas analysis has been reported to have a sampling failure rate of 20.6% as compared to 1.2% for lactate measurement (Westgren *et al.*, 1998). Lactate is the major component of metabolic acidosis which develops in the late stage of fetal hypoxia when there is an insufficient supply of oxygen. Both animal (Milley, 1988) and human studies (Piquard *et al.*, 1991; Nordström *et al.*, 2001) have suggested that umbilical cord blood lactate is predominantly fetal in origin but minor transfer from the mother to fetus cannot be excluded. Umbilical cord blood lactate levels have been shown to correlate with the pH and BE (Gjerris *et al.*, 2008).

Several studies have investigated the influence of sampling site and time interval after delivery on umbilical cord blood gas levels and acid-base status (Sykes and Molloy, 1984; Duerbeck *et al.*, 1992; Armstrong and Stenson,

2006; Lynn and Beeby, 2007). From where the blood is drawn is important, as samples from a double or single clamped umbilical cord yield different results. For example Lynn and Beeby found, in paired samples, that mean pH and BE levels were significantly lower in arterial bloods from double clamped cords at the time of delivery of the infant, compared to bloods from surface arteries of the subsequently delivered placenta and whose cords were effectively single clamped (Lynn and Beeby, 2007). The cord arterial pH fell steadily and significantly from delivery levels (at 30, 60 and 90 minutes) and pH levels from the placental surface artery also fell significantly over 90 minutes, but at twice the rate (Lynn and Beeby, 2007). Armstrong and Stenson, in paired arterial samples, found that mean pH levels from 'unclamped' umbilical cord samples were significantly higher than from clamped umbilical cord samples at delivery; but found no differences between paired mean venous pH levels in clamped or unclamped cord samples (Armstrong and Stenson, 2006). (Unclamped was defined as from a vessel at the base of the cord that remained in continuity with the placenta and before the first clamp (Armstrong and Stenson, 2006).)

The change over time of pH and BE levels in blood drawn from a single clamped umbilical artery compared to those from a double clamped umbilical artery appears to be inconsistently reported in the literature. For instance, Lynn and Beeby found that the arterial pH and BE levels were significantly reduced at 30 minutes post-delivery in both the placental surface artery (effectively single clamped) and double clamped cord samples (Lynn and Beeby, 2007). Whereas Armstrong and Stenson reported a significant

reduction in mean arterial pH at 40 minutes post-delivery in unclamped arteries (effectively single clamped) but no differences over 60 minutes in double clamped arteries (Armstrong and Stenson, 2006). Significant changes in mean arterial BE levels were seen at 20 minutes post-delivery in unclamped arteries and in 40 minutes in double clamped arteries (Armstrong and Stenson, 2006).

Results can also differ when cord blood samples are analysed from apparently comparable sites. For instance, in studies which sampled from double clamped sections of cord, the arterial pH levels reduced significantly, though modestly, from delivery in 10 minutes (Paerregaard *et al.*, 1987), in 30 minutes (Lynn and Beeby, 2007), and not at all (i.e. up to 60 minutes) (Armstrong and Stenson, 2006). Mean BE levels in double clamped arterial cord samples showed no change over 30 minutes in one study (Paerregaard *et al.*, 1987), but a significant reduction at 30 minutes in a different study (Lynn and Beeby, 2007), and also at 40 minutes (Armstrong and Stenson, 2006). In double clamped venous cord blood, significant reductions in pH levels were found at 10 minutes (Paerregaard *et al.*, 1987), and at 40 minutes (Armstrong and Stenson, 2006). In studies which sampled from single clamped cord (referred to variously as unclamped, placental artery or single clamped samples), arterial pH levels were significantly reduced from delivery by 30 minutes (Lynn and Beeby, 2007) and 40 minutes (Armstrong and Stenson, 2006). Single clamped venous cord pH levels is less reported in the literature but in one study pH levels were significantly reduced from delivery at 40 minutes (Armstrong and Stenson, 2006). In our study, which

also used venous samples from a single clamped umbilical cord, we found a significant change in pH and BE levels within seven minutes post-delivery.

The reason(s) for the discrepancy in the time taken for the pH and BE levels to fall between studies is not clear. Most of the studies were relatively small; the largest study had between 51-87 pairs of samples. The sampling time intervals for pH and BE measurements, both from single and double clamped samples, were often too wide at 20 or 30 minute intervals, to allow accurate timing of changes in levels (e.g. Armstrong and Stenson, 2006; Lynn and Beeby, 2007). Differences in the way samples are handled prior to sampling also may affect the rate of change in levels. For example storing blood in heparinised syringes, on ice (i.e. 0°C) has been shown to prevent significant decreases in pH levels over 30 minutes; whereas there are significant albeit small decreases if stored at room temperature (Pel and Treffers, 1983).

In Ninewells Hospital and Medical School, umbilical cord blood sampling post-delivery is normally carried out by the midwife who has cared for the mother, but immediate sampling sometimes may not be possible especially when a midwife's attention is focused on resuscitation of the infant. A normal metabolic status at delivery, as assessed by cord blood sample estimations of pH, BE and lactate levels, can rapidly become classified as abnormal simply as a result of delayed blood sampling and/or delayed sample analysis. These conclusions are very relevant to the metabolic and hormonal studies planned for this study and underline the importance of the need for as rapid as possible blood sampling. It is possible, therefore, in the

first pilot study in the series reported here that there may have been delays in collection and/or analysis of the cord blood samples which resulted in glucose consumption and hence giving an artefactually high incidence of hypoglycaemia in infants delivered by elective caesarean section. In that first study, information was not recorded on the time interval from delivery of the infant to analysis of the corresponding cord venous blood sample. To confirm whether or not the incidence of hypoglycaemia in our sample of elective caesarean sections was likely to be valid, a further study was undertaken to determine the time interval between delivery and first blood sample analysis in routine clinical practice at Ninewells Hospital and Medical School, Dundee (pilot study 3).

CHAPTER 4

PILOT STUDY 3: TIME INTERVAL FROM DELIVERY TO UMBILICAL CORD BLOOD GAS AND ACID-BASE STATUS MEASUREMENT: ROUTINE CLINICAL PRACTICE

4.1 INTRODUCTION

The purpose of this study was to record the time interval from delivery of the infant to the analysis of the first cord blood sample taken for routine blood gas and acid-base status after spontaneous vaginal deliveries and following elective caesarean sections.

4.2 METHODS

4.2.1 Mothers and infants

The clinical records of 100 consecutive women who had a spontaneous vaginal delivery and 100 consecutive women who had an elective caesarean section in the period April to May 2005 were identified retrospectively using the “Protos” database. Protos is an electronic system where all deliveries in the Tayside Region are recorded and information for each patient is entered by midwives and obstetricians at the time of delivery. The time of birth and that of the delivery of the placenta were identified from the delivery summary of each individual included in this study from their Protos record; the time of analysis of the respective cord blood gas levels and acid-base status were obtained from the AVL OMNI Modular System blood gas analyser (Roche Diagnostics GmbH, Graz, Austria) located in the Labour Suite which electronically stored all the records and the time of analysis of individual

blood samples. The times recorded by Protos and that recorded by AVL OMNI Modular System blood gas analyser were accurate within \pm two minutes. No ethical approval was required for this study.

4.2.2 Statistical analyses

Data were entered into Microsoft Excel XP for analysis. The means and SD of the time taken from delivery to measurement of blood gas levels and acid-base status, as well as the time taken from delivery of the placenta to measurement of blood gases were calculated for both the spontaneous vaginal delivery and caesarean section groups.

4.3 RESULTS

One hundred consecutive cases were identified each of spontaneous vaginal deliveries and of elective caesarean sections. The mean time taken to obtain an umbilical blood gas was 17 minutes following caesarean section and 30 minutes after delivery of the infant by spontaneous vaginal delivery (Table 4.1). After the delivery of the placenta, the mean time taken was 15 minutes for the caesarean section group compared to 23 minutes in the spontaneous vaginal group (Table 4.1).

Table 4.1: Time taken to measure blood gases after delivery: routine clinical care

	Elective caesarean section n=100 Mean \pm SD (range)	Spontaneous vaginal delivery n=100 Mean \pm SD (range)
After second stage (min)	17 \pm 6.40 (7-53)	30 \pm 14.00 (13-109)
After third stage (min)	15 \pm 6.36 (5.52)	23 \pm 15.00 (4-101)

4.4 COMMENTARY

Umbilical cord blood gas and acid-base status is considered to be a more objective measure of the condition of the infant at birth than Apgar scores, and abnormal results may indicate the occurrence of an acute intra-partum hypoxic event with consequent medico-legal implications. Some studies recommend routine cord blood sampling and analysis for all deliveries (Thorp *et al.*, 1989; Johnson *et al.*, 1990) whereas others suggested selective sampling of at risk populations (Duerbeck *et al.*, 1992). In the UK, guidelines from the National Institute for Health and Clinical Excellence (NICE) recommend that “paired [arterial and venous samples] cord blood gases do not need to be taken routinely. They should be taken when there has been concern about the baby either in labour or immediately following birth” (NICE, 2007). In contrast, the American College of Obstetricians and Gynecologists (ACOG) recommends taking paired arterial and venous cord blood samples from: all infants delivered by caesarean section for fetal compromise; those infants with low five minute Apgar scores, severe growth restriction or abnormal fetal heart rate tracings; or when there is maternal thyroid disease, intrapartum fever or a multiple birth (ACOG Committee on Obstetric Practice, 2006). A recent systematic review and meta-analysis suggested that there are strong associations between low umbilical cord

artery pH at birth and neonatal mortality, hypoxic ischaemic encephalopathy, intraventricular haemorrhage or periventricular leucomalacia, and cerebral palsy (Malin *et al.*, 2010).

In 1994, routine sampling of umbilical arterial and venous cord blood for estimation of pH, pO₂, pCO₂, cHCO₃ and BE for all infant deliveries was introduced into routine practice in Ninewells Hospital and Medical School, Dundee. However there was no written protocol for umbilical cord blood sampling procedures although it was expected that the midwife who attended the birth would sample and analyse the blood as soon as possible after delivery. Neither the RCOG nor Royal College of Midwives (RCM) have produced any guidelines on the technique of umbilical cord blood sampling. There is no protocol for this procedure in the labour ward in Ninewells Hospital and Medical School, despite the acceptance of routine umbilical blood gas and acid-base status analysis being the norm for all deliveries since 1994. In general, midwives in the labour ward have been taught not only to obtain umbilical blood gases but also to analyse the sample using a blood gas analyser within the labour ward. The general assumption is that this sampling and analysis is done as soon as possible after deliveries but there is no time interval specified, nor guidance on where the blood samples should be obtained i.e. for example either from the umbilical cord or placenta insertion. In addition, there is no guidance on the technique of isolation of a segment of cord through double-clamping which is recommended by the ACOG (ACOG Committee on Obstetric Practice, 2006). The ACOG has also recommended that an arterial umbilical cord blood sample is the preferred

blood sample, but where possible, and ideally, both a venous and arterial sample (paired specimen) should be collected and analysed; it is further emphasized that it is important to label the samples as either venous or arterial. The validity of the latter point has been emphasised by Tong and colleagues who have reported that 53 out of 289 (18.3%) supposedly paired umbilical arterial and venous samples were invalid; of the 53 samples, nine had been incorrectly labelled (Tong *et al.*, 2002). Paired samples are normally taken by the midwives in our labour ward but unfortunately the samples are generally not routinely labelled as arterial or venous; when there is only one blood sample obtained, it is sometimes difficult in retrospect to determine whether it is an arterial or venous sample.

The sampling and subsequent analysis of umbilical cord blood samples needs to be a simple and fast procedure, but immediate sampling and analysis of cord blood sometimes may not be possible, especially if a midwife's attention is focused for example on resuscitation of an infant. Our study showed that the mean time taken to obtain an umbilical blood gas was 17 minutes after delivery of the infant by caesarean section and 30 minutes after delivery of the infant by spontaneous vaginal delivery. In one extreme case, a sample was taken 109 minutes after spontaneous vaginal delivery of an infant. A normal metabolic status at delivery, as assessed by cord blood sample estimations of pH and BE, can rapidly become classified as abnormal simply as a result of delayed blood sampling and/or delayed sample analysis. When we looked at the temporal changes in cord blood gas, acid base status, glucose and lactate levels with time (pilot study 2), we

found that the glucose level had changed significantly by 12 minutes whereas lactate, pH and BE levels had changed significantly as early as seven minutes. Both studies suggest that infants delivered by spontaneous vaginal deliveries are at a higher risk of hypoglycaemia. Surprisingly, when we measured the incidence of hypoglycaemia (pilot study 1) we found a higher incidence of hypoglycaemia in infants delivered by elective caesarean sections than in infants delivered by spontaneous vaginal deliveries. However, the numbers of the infants delivered by elective caesarean section in this sample (n=37) whom we found to be hypoglycaemic were relatively small (n=13). A combination of delay in blood sampling and/or analysis could easily account for the apparent contradictory findings. Because of the potential importance of unrecognised hypoglycaemia in term infants, and using the methodological knowledge gained during the pilots, we designed a study to describe the hormonal and metabolic profiles of term infants delivered by elective caesarean section.

CHAPTER 5

**STUDY 4: METABOLIC AND HORMONAL RESPONSES IN
THE REGULATION OF BLOOD GLUCOSE LEVELS
IN INFANTS DELIVERED BY
ELECTIVE CAESAREAN SECTION**

5.1 INTRODUCTION

The results of the three pilot studies clarified the methodology for the main study. The key methodological concept derived from these pilot studies was the importance of prompt sampling of cord blood and prompt analysis of samples; as some hormones and metabolites, such as glucose, lactate and catechols, changed significantly with time. The aim of the main study was to describe the hormonal and metabolic profiles of 'unstressed' term infants delivered by elective caesarean section. Women who planned for an elective caesarean section were chosen in the light of the results from the pilot study 2 and 3, which showed possible reasons for the highest proportion of hypoglycaemic infants were delivered by elective caesarean section in the pilot study 1. Furthermore in terms of practicality, it was easier to recruit and to obtain umbilical cord samples within five minutes of delivery of these infants. The objectives have been listed in the introduction chapter (see page 35).

5.2 METHODS

5.2.1 Mothers and infants

The study population were women undergoing elective caesarean section in Ninewells Hospital and Medical School, Dundee, at 37+ weeks gestation with a singleton pregnancy. Women were recruited into the study between July 2004 and April 2006. The eligibility criteria and recruitment procedures were the same as those for pilot study 2 (see page 37 and Appendix for Information Sheets, pages 156-157).

Analysis of the first pilot study's data showed that a lower umbilical venous pO_2 was significantly associated with hypoglycaemia. This was used to determine the sample size. Using a ratio of two controls (i.e. normoglycaemic) to one case (i.e. hypoglycaemic), a sample size comprising 96 controls and 48 cases would have sufficient power ($\alpha = 5\%$, $\beta = 80\%$) to detect a change in mean value of pO_2 between normo- and hypoglycaemic groups of 0.5kPa.

5.2.2 Blood sample collection

All the blood samples were taken by me. A venous blood sample for glucose and lactate estimations was taken from a maternal foot vein using a 21 gauge butterfly needle (Hospira, Lake Forest, USA) immediately after delivery of the infant. The reason for taking a blood sample from a foot vein was to reduce any discomfort to the mother as the majority had spinal anaesthesia for the delivery. Blood samples for glucose and lactate estimations were collected into fluoride-oxalate tubes before transportation to

the Department of Biochemical Medicine, Ninewells Hospital and Medical School, Dundee for analysis.

Approximately 20 ml of blood was taken from the umbilical vein at the point of placental cord insertion (singly-clamped sample) using a 19 gauge butterfly needle (Abbott Ireland, Sligo, Republic of Ireland) and aliquoted within five minutes of delivery into a variety of tubes according to the analyte to be measured. Venous cord blood was assayed for glucose, lactate, 3-hydroxybutyrate, FFA, amino acids (alanine, arginine, citruline, cystine, GABA, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine ornithine, phenyalanine, serine, taurine, threonine, tyrosine and valine), insulin, glucagon, hGH, cortisol, catechols (EPI, NE, DA, DOPA, DHPG, DOPAC) and their sulfated conjugates (EPI sulfate, NE sulfate, DA sulfate, DOPA sulfate, DHPG sulfate, DOPAC sulfate), pH, pO₂, pCO₂, cHCO₃ and BE. Blood for glucose, lactate, 3-hydroxybutyrate and FFA estimations was collected into fluoride-oxalate tubes. Blood for analysis of amino acids, insulin, glucagon, hGH, cortisol and catechols was collected into lithium heparin tubes and placed onto ice; they were sent immediately to the Department of Biochemical Medicine, Ninewells Hospital and Medical School, Dundee for plasma preparation and subsequent assaying. Blood samples for blood gas levels and acid-base status were collected using one ml heparinised plastic syringes (BD Diagnostic, Plymouth, UK). Blood gas levels and acid-base status were analysed in the AVL OMNI Modular System blood gas analyser (Roche Diagnostics GmbH, Graz, Austria) located in the labour ward and serviced by the Department of Biochemical Medicine,

Ninewells Hospital and Medical School as part of the UK national accreditation scheme.

Venous cord blood was the preferred source for this study as the majority of hormonal and metabolites research studies to date had used umbilical venous samples to measure hormones and metabolites at birth (see Chapter 1). There were also practical considerations as large volumes of blood would be required for measurement for the range of hormones and metabolites as mentioned above. This would enable a range of analytes measured for each infant as opposed to many other studies which were only able to measure one or two analytes. Sampling from the umbilical artery is more challenging than from the vein as the artery has smaller lumen and contains less blood.

5.2.3 Data collection

The core epidemiological data were abstracted from the maternal case notes, as described for pilot study 1, (see page 38 and Appendix for a copy of the Data Abstraction Form, pages 158-166). Additional data were collected, including delivery weight and height of the mother to calculate BMI (delivery BMI was felt to be more physiologically appropriate to investigate the impact of delivery on the fetal hormonal and metabolic responses in the regulation of blood glucose levels); drugs, fluid (volume and type) and analgesia received during the preoperative period; and the reason for the elective caesarean section. All data were double-entered into Epi Info™

version 3.4.3 by an experienced data manager. The data were exported from Epi Info™ to SPSS for statistical analysis.

5.2.4 Statistical analyses

SPSS version 14 statistical software was used to conduct all analyses. Means and SDs were calculated for levels of glucose, lactate, 3-hydroxybutyrate, FFA, amino acids, insulin, glucagon, hGH, cortisol, catechols and their sulfated conjugates, pH, pO₂, pCO₂, and cHCO₃ and BE. Statistical significance was determined, as appropriate, by linear regression analysis, Student's two tailed *t*-test, or Chi-square test.

The analysis of the data was done in two steps using univariate general linear modelling. First, the impact of each categorical and continuous variable was assessed singly upon the levels of glucose and lactate. Secondly, factors that were significantly associated as well as those supported by literature were entered together into a final model to determine their adjusted impact. All of the variables included in the first and second steps are listed in Table 5.6 and Table 5.7 respectively (see Appendix pages 141-143). The one sample Kolmogorov-Smirnov test was used to evaluate the normality of the distribution. Data that were not normally distributed were log transformed before analysis. Statistical significance was taken at *p* value ≤ 0.05 .

5.2.5 Biochemical analyses

5.2.5.1 Glucose (mmol/l)

The analysis of glucose levels was identical to that described for the first pilot study (see pages 40-41 for details).

5.2.5.2 Lactate (mmol/l)

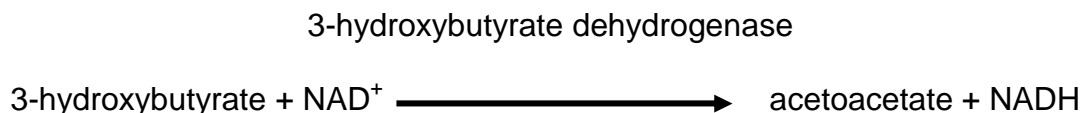
The analysis of lactate levels was identical to that described for the first pilot study (see pages 41-42 for details).

5.2.5.3 3-Hydroxybutyrate (mmol/l)

3-hydroxybutyrate concentrations were measured at the Metabolic Biochemistry laboratory of the Royal Hospital for Sick Children, Edinburgh using a Randox kit (Randox Laboratories Ltd, Antrim, United Kingdom) on a Roche Modular P Analyser (Roche Diagnostics GmbH, Mannheim, Germany). Venous blood samples were collected into fluoride-oxalate tubes. Following centrifugation at 4000 g for five minutes at 4°C, the resulting plasma was immediately frozen and stored at -20°C in the laboratory freezer in the Department of Biochemical Medicine, Ninewells Hospital and Medical school. The samples remained frozen until analysis at the Metabolic Biochemistry laboratory of the Royal Hospital for Sick Children, Edinburgh.

The method of analysis is based on the reaction which converts 3-hydroxybutyrate to acetoacetate using 3-hydroxybutyrate dehydrogenase in the presence of nicotinamide dinucleotide (NAD). During this reaction an

equimolar amount of NAD is reduced to NADH which is measured at 340nm and is proportional to the 3-hydroxybutyrate concentration.



The production of NADH by the action of endogenous lactic dehydrogenase (LDH) on lactic acid is prevented by incorporating oxalic acid, an inhibitor of LDH activity, in the assay reagents. The assay sensitivity was 0.1 mmol/L and the inter-assay coefficient of variation was 2.4%.

5.2.5.4 Free fatty acids (mmol/l)

Free fatty acids concentrations were measured at the Metabolic Biochemistry laboratory of the Royal Hospital for Sick Children, Edinburgh using a Wako NEFA C kit (Wako Chemicals GmbH, Neuss, Germany) on a Roche Modular P Analyser (Roche Diagnostics GmbH, Mannheim, Germany). Venous blood was collected into a fluoride-oxalate tube. After centrifugation at 4000 g for five minutes at 4°C, the plasma was separated and kept frozen at -20°C in the laboratory freezer in the Department of Biochemical Medicine, Ninewells Hospital and Medical School, Dundee prior to transfer to Edinburgh for analysis. The samples remained frozen until analysis in the Metabolic Biochemistry laboratory of the Royal Hospital for Sick Children, Edinburgh.

This assay is based on enzymatic spectrophotometric techniques and relies upon the acylation of coenzyme A (CoA) by the fatty acids in the presence of

added acyl-CoA synthetase (ACS). The acyl-CoA thus produced is oxidized by added acyl-CoA oxidase (ACOD) with generation of hydrogen peroxide; in the presence of peroxidase (POD) this permits the oxidative condensation of 3-methy-N-ethyl-N(β -hydroxyethyl)-aniline (MEFA) with 4-aminoantipyrine to form a purple coloured adduct which can be measured colorimetrically at 550 nm. The assay sensitivity was 0.006 mmol/l and the inter-assay coefficient of variation was 1.1%.

5.2.5.5 Amino acids ($\mu\text{mol/l}$)

The amino acid analyses were performed using a Biochrom 20 Amino Acid Analyser (Biochrom Ltd, Cambridge, United Kingdom) in the Department of Biochemical Medicine, Ninewells Hospital and Medical School, Dundee, using a technique based on ion exchange liquid chromatography. A plasma sample containing a mixture of amino acids was loaded, automatically, onto a column of cation-exchange resin. Buffers of varying pH and ionic strength are used in the system to separate the various amino acids. The temperature of the column is also accurately controlled and can be changed at any point in the analysis cycle to produce the required separation. The column eluent is mixed with the ninhydrin reagent and the mixture passes through the high temperature reaction coil. In the reaction coil, the ninhydrin reacts with the amino acids present in the eluent to form coloured compounds, which is measured using a photometer; the amount of light absorbed is directly proportional to the quantity of amino acid present. The photometer output is connected to a computer, which plots the amino acid concentrations as a series of peaks. The retention time of the peak on the chart identifies the

amino acid, the area under the peak indicates the quantity of amino acid present. As an amino acid analyser is a comparative instrument, a calibration analysis must be performed before commencing each series of analyses, to produce a standard trace for comparison purposes.

Blood samples were collected into lithium heparin tubes, placed onto ice and immediately brought to the laboratory to be spun at 4000 g for five minutes at 4°C. The minimum volume of plasma required for amino acid assay is 100 µL. The inter-assay coefficient of variation for amino acids was less than 10%.

5.2.5.6 Insulin (mU/l)

Insulin was measured using the IMMULITE®2000 Insulin kit (Euro/DPC Ltd, Gwynedd, United Kingdom) on an IMMULITE®2000 analyser (Euro/DPC Ltd, Gwynedd, United Kingdom) in the Department of Biochemical Medicine, Ninewells Hospital and Medical School, Dundee. IMMULITE®2000 Insulin is a solid phase, two-site chemiluminescent immunometric assay. The solid phase which is a bead coated with murine monoclonal anti-insulin antibody, is incubated with the plasma and alkaline phosphatase conjugated sheep polyclonal anti-insulin antibody for 30 minutes at room temperature. The unbound alkaline phosphatase conjugated sheep polyclonal anti-insulin antibodies are removed by a centrifugal wash. In the final step, a chemiluminescent substrate, adamanthyl dioxetane phosphate, is added to the bead and the resulting photon emission signal is this time directly proportional to the concentration of insulin in the sample.

Blood samples were collected directly into lithium heparin tubes and transferred immediately on ice to the laboratory for plasma preparation and assaying. The minimum volume of plasma required for insulin assay is 100 μ L and the assay can measure insulin concentrations between 2 mU/l and 300 mU/l. The inter-assay coefficient of variation for insulin was 6.24%.

5.2.5.7 Glucagon (ng/l)

Blood samples were collected into lithium heparin tubes, placed onto ice and immediately brought to the laboratory to be spun at 4000 g for five minutes at 4°C. The plasma was then stored and kept frozen at -20°C in the laboratory freezer in the Department of Biochemical Medicine, Ninewells Hospital and Medical School, Dundee. All of the samples were sent by air to the Metabolic Laboratory of the Royal Victoria Hospital, Belfast, Northern Ireland for analysis. All the samples remained frozen until analysis.

The assay is based on radioimmunoassay techniques. The principle of radioimmunoassay depends on the assumption that radio-labelled and unlabelled antigen compete equally for sites on an antibody. A standard quantity of tracer radio-labelled peptide is added into the antibody-antigen system and displaced antibody-bound peptide. The quantity of bound labelled trace is inversely proportional to the quantity of peptide being assayed. There is no external quality assurance scheme for glucagon estimations and hence, there were two internally prepared control specimens in each assay. The assay had an inter-assay coefficient of variation of less than 15% and a sensitivity of 15 ng/l.

5.2.5.8 Human growth hormone (mU/l)

Human growth hormone was measured using the IMMULITE[®]2000 hGH kit (Euro/DPC Ltd, Gwynedd, United Kingdom) on an IMMULITE[®]2000 analyser (Euro/DPC Ltd, Gwynedd, United Kingdom) in the Department of Biochemical Medicine, Ninewells Hospital and Medical School, Dundee. The principle of the procedure is similar to insulin, using a solid phase, two-site chemiluminescent immunometric assay. The solid phase which is a bead coated with murine monoclonal anti-hGH antibody, is incubated with plasma and alkaline phosphatase conjugated rabbit polyclonal anti-hGH antibody for 30 minutes at room temperature. The unbound alkaline phosphatase conjugated rabbit polyclonal anti-hGH antibodies are then removed by a centrifugal wash. The amount of alkaline phosphatase conjugated rabbit polyclonal anti-hGH antibody bound is directly proportional to the concentration of the hGH in the sample. In the final step, a chemiluminescent substrate, adamantyl dioxetane phosphate, is added to the bead. In the luminogenic reaction, the adamantyl dioxetane phosphate is dephosphorylated into an unstable intermediate by the alkaline phosphatase bound to the bead. The unstable intermediate rapidly and spontaneously breaks down emitting a photon of light, which is detected by a photomultiplier tube. The amount of light emitted is directly proportional to the amount of bound alkaline phosphatase.

The blood samples were collected directly into lithium heparin tubes and placed immediately onto ice for transport to the laboratory where they were centrifuged at 4000g for five minutes. The minimum volume of plasma

required for the assay is 25 μ l and the assay could measure hGH concentrations between 0.01 mU/l and 40 mU/l. The assay had an inter-assay coefficient of variation of 4.27%.

5.2.5.9 Cortisol (nmol/l)

Plasma cortisol was measured using a Cobas[®] Cortisol kit (Roche Diagnostics GmbH, Mannheim, Germany) on a Roche Modular Serum Work Area Analyser (Roche Diagnostics GmbH, Mannheim, Germany) in the Department of Biochemical Medicine, Ninewells Hospital and Medical School, Dundee. The assay makes use of a competition test principle using a polyclonal antibody which is specifically directed against cortisol. Twenty μ l of plasma is incubated with a cortisol specific biotinylated antibody and a ruthenium complex labelled cortisol derivative. Depending on the concentration of the analyte in the sample and the formation of the respective immune complex, the labelled antibody binding site is occupied in part with sample analyte and in part with ruthenylated hapten. After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

The blood samples were collected directly into lithium heparin tubes and placed immediately onto ice prior to transport to the laboratory for immediate

analysis where they were centrifuged at 4000g for five minutes. The separated plasma is stable for 30 days at 2 – 8°C and 3 months at -20°C. The lowest and maximum detection limits for the assay were 0.5 nmol/l and 1750 nmol/l respectively. The assay had an inter-assay coefficient of variation of 8.14%.

5.2.5.10 Catechols (pg/ml)

Six plasma catechols and their respective sulfated conjugates were measured: DOPA, DOPA sulfate, DA, DA sulfate, NE, NE sulfate, EPI, EPI sulfate, DHPG, DHPG sulfate, DOPAC and DOPAC sulfate. Umbilical cord blood samples were collected into lithium heparin tubes, placed onto ice and immediately brought to the Department of Biochemical Medicine, Ninewells Hospital and Medical School, Dundee, where they were centrifuged at 4000 g for five minutes at 4°C. The resulting plasma was separated into a further tube without anticoagulant, the tube placed immediately into solid carbon dioxide pellets to freeze the contents and then stored in a -80°C freezer. All plasma samples were transported in batches by air in solid carbon dioxide to the Clinical Neurochemistry Laboratory, National Institute of Health (NIH), Bethesda, Maryland, USA. All arrived in a frozen state and were kept in a -80°C freezer until analysis.

All the catechols assays in this study were undertaken by me following training and under the supervision of Professor Graeme Eisenhofer, who is an internationally acknowledged expert in catecholamine metabolism, and who has established a laboratory with the expertise and technologies to

measure a wide range of catechols and their conjugates not currently available in the UK. The assays were done in the Clinical Neurochemistry Laboratory, NIH, Bethesda, Maryland, USA, using an alumina extraction step followed by high-performance liquid chromatography (HPLC) with electrochemical detection. The alumina extraction procedure serves to isolate and concentrate the low femtomolar concentrations of catechols in plasma into a purified low volume form appropriate for injection onto the HPLC apparatus. Alumina adsorbs catechols at alkaline pH and desorbs catechols at acidic pH. The alumina at alkaline pH was washed with water, to remove compounds other than catechols. At the end of the assay, the alumina was acidified, desorbing the catechols into the supernatant. Finally, an aliquot of the supernatant was injected into the HPLC apparatus. The function of the electrochemical detection system is to detect and quantify the analytes of interest after their separation in and elution from the analytical column. The ordered elutions from the column were analysed by measuring the currents generated from oxidation or reduction of the analytes by electrochemical analysis. The same testing principle applies for the sulfated conjugates.

The plasma was incubated with sulfatase at 37°C for 30 minutes prior to analysis. One normal level adult plasma control was used on each HPLC run. The inter-assay coefficient of variation for catechols assays in this study were 10.27% for EPI, 2.76% for NE, 6.44% for DOPA, 5.69% for DHPG, 5.47% for DOPAC, 6.16% for EPI sulfate, 13.52% for NE sulfate, 13.62% for DOPA sulfate, 11.75% for DHPG sulfate and 5.43% for DOPAC sulfate.

5.2.5.11 Blood gas and acid-base profiles

The analysis of blood gas and acid-base status was identical to that described for the first pilot study (see pages 42-43 for details).

5.3 RESULTS

One hundred and fifty three women were recruited into the study; all were scheduled for elective caesarean section. The mean maternal age and BMI at delivery of the women recruited was 32.03 years and 31.14 respectively (Table 5.1). Only a small proportion of women (13.1%) admitted to smoking during pregnancy (Table 5.1); the majority of the women were healthy with no significant past medical history and had had an uneventful pregnancy (Table 5.1).

The main reasons given for having an elective caesarean section were: previous caesarean section (65.4%), breech presentation (20.9%) and maternal request (8.5%) (Table 5.2). The majority of women had spinal or epidural anaesthesia (98.0%) for their caesarean section; only a small proportion (2.0%) had general anaesthesia (Table 5.2). The majority of women (83.7%) had at least one drug prescribed prior to delivery and some (5.9%) were prescribed up to maximum of three drugs, which included ephedrine, phenylephrine, glycopyrrolate and atropine (Table 5.2).

Only one infant was born with an Apgar score of less than three at one minute and no infant had an Apgar score of less than seven at five minutes (Table 5.2). Only one infant had an umbilical cord arterial BE greater than -

12 (Table 5.2). The mean values for cord arterial pH, BE, pO₂, pCO₂, and cHCO₃ were 7.29, -1.94, 2.29 kPa, 7.21 kPa and 25.10 mmol respectively (Table 5.3). The mean birth weight ratio, OFC and birth length of the infants were 1.08, 35.1 cm and 49.3 cm respectively (Table 5.2).

At the time of elective caesarean section, the mean maternal glucose level was 4.28 mmol/l which is within the normal range (Table 5.3). The mean maternal lactate level of 1.80 mmol/l was at the upper limit of the normal venous blood reference range (Table 5.3). Three infants had an umbilical cord blood glucose level of <2.6 mmol/l at birth. The mean infant glucose and lactate levels were, as expected for a full term infant, 3.3 mmol/l and 2.2 mmol/l respectively (Table 5.3). The mean insulin level (8.3 mU/l) and the mean glucagon level (251 ng/l) seems high. Twenty-one amino acids were measured in umbilical venous cord plasma and the mean values are summarised in Table 5.3.

Venous cord plasma glucose levels were significantly positively associated with ephedrine given to the mother prior to delivery, the reasons for caesarean section, maternal glucose, alanine, lysine, insulin, EPI and EPI sulphate, and were significantly negatively associated with the need for neonatal resuscitation and 3-hydroxybutyrate. These variables, as well as those supported by literature i.e. maternal diabetes, and levels of glucagon, hGH, cortisol and NE were entered into an univariate general model to determine their adjusted impact. Only three factors affected the umbilical venous cord glucose levels (Table 5.4). There was a significantly positive

association between maternal venous glucose level and umbilical venous cord glucose levels ($p<0.001$). A decrement of umbilical venous cord glucose levels was associated with the presence of maternal diabetes ($p<0.001$) and cord NE levels ($p=0.020$) (Table 5.4).

A much greater number of factors were found to influence the venous umbilical cord plasma lactate levels than the cord plasma glucose levels (Table 5.5). There was a significant positive association between umbilical venous cord lactate levels and maternal glucose levels ($p=0.004$), maternal lactate levels ($p<0.001$), arterial $p\text{CO}_2$ ($p<0.001$), cord glucose levels ($p=0.023$) and marginally with cord NE ($p=0.053$) levels. On the other hand there was a negative association between umbilical cord lactate levels and arterial BE ($p<0.001$) and cord glucagon levels ($p=0.014$).

Table 5.1: Characteristics of the women recruited

	Mean \pm SD (n) or N, %	Range
Maternal age, years	32.03 \pm 5.65 (153)	16.81-44.39
Maternal BMI	31.14 \pm 6.32 (150)	17.97-31.16
Maternal smokers	20/153, 13.1%	-
Antenatal events:		
Antepartum haemorrhage	3/153, 2.0%	-
Asthma	9/153, 5.9%	-
Epilepsy	1/153, 0.7%	-
Thyroid disease	2/153, 1.3%	-
Diabetes	3/153, 2.0%	-
Hyperemesis gravidarum	1/153, 0.7%	-
Tractocile treatment	1/153, 0.7%	-
Bethamethasone injection	4/153, 2.6%	-
Hypertension	3/153, 2.0%	-
Treated UTI	1/153, 0.7%	-
Treated vaginal discharge	2/153, 1.3%	-
Reasons for elective caesarean section		
Previous caesarean section	100/153, 65.4%	-
Breech presentation	32/153, 20.9%	-
Maternal request	13/153, 8.5%	-
Other reasons#	8/153, 5.2%	-
Type of analgesia received		
None received	0/153, 0.0%	-
Entonox	0/153, 0.0%	-
General anaesthesia	3/153, 2.0%	-
Epidural/spinal	150/153, 98.0%	-
Opiate	0/153, 0.0%	-
Other drugs received during delivery		
No drug	24/153, 15.7%	-
One drug	72/153, 47.1%	-
Two drugs	47/153, 30.7%	-
Three drugs	9/153, 5.9%	-

BMI body mass index, **UTI** urinary tract infection

#Other reasons included cephalopelvic disproportion, Crohn's disease, bowel problems as a result of a previous delivery, and an unstable lie.

Table 5.2: Postpartum characteristics of the infants and women recruited

	Mean \pm SD (n) or N, %	Range
Male infant	68/153, 44.4%	-
Apgar score < 3 at 1 min	1/153, 0.7%	-
Apgar score < 7 at 5 min	0/153, 0.0%	-
Cord blood arterial pH	7.29 \pm 0.05 (145)	7.039 - 7.386
Cord blood venous pH	7.33 \pm 0.05 (151)	7.047 - 7.415
Cord blood arterial BE >-12	1/148, 0.7%	-
Cord blood venous BE >-12	0/151, 0.0%	-
Cord blood arterial BE	-2.13 \pm 1.88 (145)	-12.40 - 0.80
Cord blood venous BE	-2.04 \pm 1.59 (151)	-10.40 - 0.50
Cord blood arterial pO ₂ kPa	2.15 \pm 0.72 (145)	0.33 - 4.97
Cord blood venous pO ₂ kPa	2.88 \pm 0.69 (140)	1.01 - 4.44
Cord blood arterial pCO ₂ kPa	7.21 \pm 1.01 (145)	4.68 - 10.58
Cord blood venous pCO ₂ kPa	6.30 \pm 1.34 (151)	2.48 - 16.24
Cord blood arterial cHCO ₃ mmol/l	25.10 \pm 2.35 (145)	15.00 - 29.80
Cord blood venous cHCO ₃ mmol/l	24.05 \pm 1.82 (151)	15.00 - 28.00
Neonatal resuscitation required	7/152, 4.6%	-
Infant birth weight grams	3581 \pm 527 (153)	2380 - 4900
Infant birth weight ratio	1.08 \pm 0.15 (153)	0.70 - 1.63
Infant OFC cm	35.10 \pm 1.70 (149)	23.40 - 39.00
Infant birth length cm	49.30 \pm 2.30 (149)	44.20 - 57.00

BE base excess, **cHCO₃** bicarbonate, **OFC** occipital-frontal head circumference

Table 5.3: The mean values, standard deviation and range of maternal glucose and lactate levels in peripheral venous plasma, and various hormones and metabolites in umbilical venous cord blood of term infants at birth

	Mean \pm SD (n)		Range
Maternal glucose mmol/l	4.28 \pm	0.56 (153)	3.2 - 7.3
Maternal lactate mmol/l	1.80 \pm	0.49 (153)	0.8 - 3.4
Glucose mmol/l	3.3 \pm	0.5 (153)	2.0 - 5.4
Lactate mmol/l	2.2 \pm	1.0 (153)	0.8 - 9.5
3-hydroxybutyrate mmol/l	0.18 \pm	0.14 (148)	0.01 - 0.79
FFA mmol/l	0.10 \pm	0.04 (148)	0.03 - 0.21
Amino acids μ mol/l			
Alanine	353.16 \pm	65.92 (150)	205 - 543
Aminobutyric acid	20.39 \pm	7.06 (150)	7 - 45
Arginine	90.92 \pm	26.76 (150)	16 - 230
Citrulline	18.41 \pm	5.40 (146)	10 - 39
Cystine	27.66 \pm	16.80 (146)	0 - 84
GABA	60.89 \pm	40.48 (150)	17 - 221
Glutamic acid	66.21 \pm	45.42 (150)	7 - 266
Glutamine	609.49 \pm	213.42 (150)	256 - 1425
Glycine	257.03 \pm	36.54 (149)	174 - 376
Histidine	122.88 \pm	19.79 (150)	78 - 194
Isoleucine	65.46 \pm	10.09 (150)	28 - 96
Leucine	125.10 \pm	17.71 (150)	87 - 177
Lysine	343.58 \pm	54.15 (150)	131 - 479
Methionine	37.37 \pm	10.92 (150)	10 - 88
Ornithine	63.59 \pm	10.31 (150)	42 - 96
Phenylalanine	74.39 \pm	11.45 (150)	50 - 125
Serine	133.97 \pm	17.42 (150)	95 - 182
Taurine	172.95 \pm	94.31 (150)	67 - 656
Threonine	271.76 \pm	59.55 (150)	150 - 508
Tyrosine	68.58 \pm	12.88 (150)	40 - 120
Valine	217.89 \pm	30.19 (150)	151 - 310
Insulin mU/l	8.3 \pm	5.0 (149)	1 - 39
Glucagon ng/l	251.0 \pm	55.0 (142)	140 - 460
Insulin:Glucagon ratio	0.033 \pm	0.018 (138)	
hGH mU/l	35.7 \pm	1.2 (152)	6.8 - 135
Cortisol nmol/l	139.0 \pm	49.0 (153)	38 - 289
EPI pg/ml	83.8 \pm	314.5 (142)	3 - 3096
EPI sulfate pg/ml	28.9 \pm	34.83 (144)	2 - 316
NE pg/ml	763.0 \pm	2038.0 (150)	26 - 19437
NE sulfate pg/ml	510.3 \pm	389.0 (150)	217 - 4740
DOPA pg/ml	2746.0 \pm	674.0 (150)	1665 - 8508
DOPA sulfate pg/ml	331.0 \pm	96.0 (150)	144 - 713
DHPG pg/ml	799.0 \pm	422.0 (150)	453 - 4467
DHPG sulfate pg/ml	396.0 \pm	123.0 (150)	167 - 1077

Table 5.3: continuation from above table

	Mean \pm SD (n)	Range
DOPAC pg/ml	1748.0 \pm 454.0 (149)	961 -4108
DOPAC sulfate pg/ml	270.0 \pm 102.0 (105)	95 - 778

FFA free fatty acids, **hGH** human growth hormone, **EPI** epinephrine, **NE** norepinephrine,
DOPA dihydroxyphenylalanine, **DHPG** dihydroxyphenylglycol, **DOPAC**
dihydroxyphenylacetic acid

Table 5.4: Maternal and infant factors that influence the umbilical cord venous plasma glucose level

Glucose	Effect estimate	SE	t-statistics	95% CI	p value
Ephedrine received	0.0630	0.0442	1.424	-0.0248, 0.1507	0.157
Neonatal resuscitation required	-0.0715	0.1038	-0.690	-0.2776, 0.1343	0.492
Reason for C/S					
Breech presentation	-0.0553	0.0560	-0.987	-0.1664, 0.0558	0.326
Maternal request	0.0899	0.8320	1.080	-0.0752, 0.2549	0.283
Other reasons#	-0.0959	0.0920	-1.042	-0.2785, 0.0867	0.300
Previous C/S	Reference				
Maternal diabetes	-2.6868	0.2661	-10.096	-3.2148, -2.1587	<0.001***
Maternal glucose mmol/l	0.7615	0.0565	13.488	0.6495, 0.8736	<0.001***
3-hydroxybutyrate mmol/l	-0.0203	0.1548	-0.131	-0.3275, 0.2870	0.896
Alanine µmol/l	0.0003	0.0004	0.694	-0.0005, 0.0010	0.489
Lysine µmol/l	-0.0001	0.0004	-0.129	-0.0009, 0.0008	0.898
Insulin mU/l	-0.0055	0.0055	-1.004	-0.0165, 0.0054	0.318
Glucagon ng/l	0.0007	0.0004	1.863	-4.800e-5, 0.0015	0.065
hGH mU/l	-0.0021	0.0012	-1.766	-0.0044, 0.0003	0.080
Cortisol nmol/l	-0.0001	0.0005	-0.218	-0.0011, 0.0009	0.828
EPI pg/ml	0.0007	0.0005	1.332	-0.0003, 0.0017	0.186
EPI sulfate pg/ml	0.0019	0.0013	1.512	-0.0006, 0.0044	0.134
NE pg/ml	-0.0001	0.0001	-2.373	-0.0002, -0.0002	0.020*

Adjusted R squared = 0.763

*p<0.05, ** p<0.01, ***p<0.001

C/S cesarean section, **hGH** human growth hormone, **EPI** epinephrine, **NE** norepinephrine

#Other reasons included cephalopelvic disproportion, Crohn's disease, bowel problems as a result of a previous delivery, and an unstable lie.

Table 5.5: Maternal and infant factors that influence the umbilical cord venous plasma lactate level

Log lactate	Effect estimate	SE	t-statistics	95 % CI		p value
Maternal glucose mmol/l	0.0552	0.0185	2.978	-0.0920,	-0.0183	0.004**
Maternal lactate mmol/l	0.0918	0.0168	5.460	0.0584,	0.1252	<0.001***
Apgar 5 min	-0.0142	0.0176	-0.810	-0.0491,	0.0207	0.420
Arterial BE	-0.0192	0.0051	-3.731	-0.0294,	-0.0090	<0.001***
Arterial pCO ₂ kPa	0.0342	0.0075	4.535	0.0191,	0.0491	<0.001***
Cord glucose	0.0531	0.0228	2.320	0.0076,	0.0985	0.023*
Alanine µmol/l	0.0001	0.0002	1.161	-0.0001,	0.0005	0.249
Isoleucine µmol/l	-0.0008	0.0010	-0.758	-0.0028,	0.0012	0.451
Leucine µmol/l	0.0010	0.0006	1.610	-0.0003,	0.0023	0.111
Tyrosine µmol/l	-0.0006	0.0007	-0.797	-0.0020,	0.0008	0.427
Valine µmol/l	-0.0005	0.0003	-1.575	-0.0012,	0.0001	0.119
Insulin mU/l	-0.0018	0.0018	-1.008	-0.0055,	0.0018	0.316
Glucagon ng/l	-0.0003	0.0001	-2.498	-0.0006,	-6.855E-5	0.014*
hGH mU/l	0.0005	0.0004	1.404	-0.0002,	0.0013	0.164
Cortisol nmol/l	0.0001	0.0002	0.642	-0.0002,	0.0004	0.523
EPI pg/ml	9.602E-5	0.0002	0.420	-0.0004,	0.0006	0.675
EPI sulfate pg/ml	0.0006	0.0004	1.450	-0.0002,	0.0015	0.151
NE pg/ml	3.789E-5	0.0001	1.962	-5.031E-7,	7.629E-5	0.053*
NE sulfate pg/ml	1.002E-5	0.0001	0.138	-0.0001,	0.0002	0.891
DOPA pg/ml	-3.592E-7	0.0001	-0.032	-2.247E-5,	2.175E-5	0.974
DOPA sulfate pg/ml	4.883E-5	0.0001	0.570	-0.0001,	0.0002	0.570
DHPG pg/ml	4.657E-5	0.0001	0.706	-8.496E-5,	0.0002	0.482
DHPG sulfate pg/ml	-0.0001	0.0001	-1.172	-0.0003,	8.015E-5	0.244

Adjusted R squared = 0.752

*p<0.05, ** p<0.01, ***p<0.001

hGH human growth hormone, **EPI** epinephrine, **NE** norepinephrine, **DHPG** dihydroxyphenylglycol, **DOPA** dihydroxyphenylalanine

5.4 COMMENTARY

5.4.1 Maternal and fetal glucose homeostasis

Only three infants from the group of 153 infants (1.96%) had an umbilical blood glucose level of less than 2.6 mmol/l at birth compared to 13 out of 37 infants (35%) from pilot study 1. In retrospect, this result was not entirely surprising as the samples in this main study, unlike the first pilot, were taken in a very controlled and timely manner – following the lessons learned in pilot studies 2 and 3. Umbilical cord glucose levels decreased over time and were significantly lower at 12 minutes compared to the baseline levels at two minutes post- delivery of infants ($p < 0.01$) (see Chapter 3). The high incidence of hypoglycaemia found in pilot study 1 was likely due to error introduced by the small number of samples, as well as the delay in sampling.

The mean umbilical glucose level in these elective caesarean section infants of the current study was lower than the mean umbilical glucose levels of infants delivered by spontaneous vaginal delivery, breech vaginal delivery, operative vaginal delivery or emergency caesarean section from pilot study 1. Labour is a stressful event for the fetus but this degree of stress at birth is probably beneficial. It is widely established that infants who delivered by elective caesarean section have higher respiratory morbidity compared to infants delivered vaginally (e.g. Zanardo *et al.*, 2004; Hansen *et al.*, 2008). Labour may have a beneficial effect on the fetal blood glucose levels. Infants delivered vaginally have higher levels of cortisol (Mears *et al.*, 2004; Miller *et al.*, 2005), EPI and NE (e.g. Irestedt *et al.*, 1982; Wang *et al.*, 1995), and lower insulin levels (Wang *et al.*, 1995) compared to infants who delivered by

elective caesarean section. Levels of cortisol, EPI and NE are known to increase blood glucose levels through various mechanisms. Cortisol exerts hyperglycaemic actions by increasing lipolysis and gluconeogenesis, as well as by inhibiting glucose uptake and utilisation by the muscle (Ganong, 1995b). EPI and NE cause an increase in blood glucose level by increasing gluconeogenesis in the liver, inhibiting glucose disposal by insulin-dependent tissues such as skeletal muscle, and by increasing gluconeogenic precursors e.g. lactate, alanine and glycerol (Ganong, 1995b). Also, both EPI and NE are inhibitors of insulin secretion (Ganong, 1995b).

Could fasting in women who had caesarean sections have had any impact on the blood glucose in the infants? Fetal glucose level is closely related to maternal glucose level (Economides *et al.*, 1989). During labour women are allowed to have a light diet, but those who plan a caesarean section are required to fast for at least six hours. In this current study, all women fasted for an average of 13.5 hours prior to delivery by elective caesarean sections. The mean maternal glucose level was 4.28 mmol/l which is within the normal non-pregnant range for adults but the levels could be lower compared to other groups of women who had other mode of deliveries. A randomised controlled trial by Scrutton and colleagues (Scrutton *et al.*, 1999) assessed the risks and benefits of eating in labour. The trial found that plasma glucose was significantly lower in the starved group (4.58 mmol/l) compared to the group of women, who were allowed to have a light diet (5.20 mmol/l) in labour at the end of an average of 11-12 hours of first stage of labour (Scrutton *et al.*, 1999). The mean and standard deviation of time between

last maternal meal and delivery of the infant was 13.21 ± 2.36 hours. Univariate analysis of the current study did not show an association between umbilical glucose levels and the time from the last maternal meal until delivery of the infant. Therefore as the time between food consumption and delivery in this study resulted in a relatively short period of fasting in the women, it is not a likely explanation of the lower blood glucose levels in infants delivered by elective caesarean section observed in this study.

The current study showed a significant positive association between maternal plasma glucose and umbilical cord venous glucose levels. This is not surprising. Studies in pregnant sheep have demonstrated a direct relationship of both fetal glucose uptake and placenta glucose consumption to the maternal blood glucose levels (Hay and Mezmarich, 1989). A study by Economides and colleagues (Economides *et al.*, 1989) on the levels of glucose and insulin in fetal blood obtained by cordocentesis between 17 to 38 weeks gestation suggested that the major determinant of blood glucose levels in the fetus is the maternal blood glucose level.

The current study has shown that umbilical cord venous glucose levels are lower than maternal venous glucose levels. This is consistent with the results shown by Kalhan and colleagues, who studied the source of human fetal glucose in ten pregnant women who had undergone elective caesarean section at term. They concluded that during intrauterine life, the maintenance of glucose levels in the human fetus is dependent upon the maternal supply of glucose (Kalhan *et al.*, 1979). A study of sheep has

confirmed that glucose passes from the maternal circulation to the fetal circulation down a concentration gradient (Meschia *et al.*, 1980). It is technically and practically very challenging to confirm this in a human study as it would require measurements of maternal uterine artery and venous glucose levels at the time of caesarean section. However using a tracer study, Prendergast and colleagues studied glucose metabolism in the human placenta and fetus at term by infusing the stable isotope, 6,6-²H₂ glucose, continuously to 14 patients during elective caesarean section. This study showed that the glucose levels were lower in the uterine vein than in the maternal artery (arterialized peripheral vein) (Prendergast *et al.*, 1999).

5.4.2 Lactate and glucose homeostasis in infants

The mean umbilical venous cord lactate level of 2.2 mmol/l found in the current study is comparable to that reported by Armstrong and Stenson of 2.51 mmol/l (Armstrong and Stenson, 2006), and Shirey and colleagues of 2.52 mmol/l (Shirey *et al.*, 1996) but is higher than that reported by Westgren and colleagues of 1.44 mmol/l (Westgren *et al.*, 1998). My study used the wet chemistry method (Roche Modular Serum Work Area Analyser), which uses plasma to measure the lactate levels whereas the other three studies used the strip method, which uses whole blood to measure the lactate levels in umbilical cord venous. Lactate concentrations are higher in the plasma compared to in the whole blood (Foxdal *et al.*, 1990). Hence different methods of analysis cannot explain the differences in findings, as the levels from my study would be expected to be higher than those of Armstrong and Stenson (Armstrong and Stenson, 2006) and Shirey (Shirey *et al.*, 1996).

There are still no agreed normal reference ranges for lactate levels for newborn populations. A level of more than 4.8 mmol/l has been suggested as an indicator of fetal asphyxia (Krüger *et al.*, 1999). However, a recent study by Wiberg and colleagues which analysed nearly 18,000 umbilical arterial and venous blood lactate levels of 'vigorous' newborns, and who had a five-minute Apgar score corresponding to the gestational age specific median value minus one point score or better at birth, suggested using gestational age-dependent lactate reference values instead of a stationary cut-off as they found that lactate levels increased with gestational age (Wiberg *et al.*, 2008).

In the current study, cord lactate levels were positively and significantly associated with maternal lactate levels. The umbilical cord lactate levels were also significantly higher than that of the mother, a finding in accord with the study carried out by Suidan and colleagues (Suidan *et al.*, 1984). The fetal-maternal difference is even greater in vaginally delivered infants (Suidan *et al.*, 1984). Like glucose, lactate has been shown to be an important substrate for fetal metabolism (Fisher *et al.*, 1980). In normal fetal lambs' hearts, lactate accounts for nearly 40% of myocardial oxygen consumption (Bartelds *et al.*, 2000). Studies in gravid sheep have demonstrated that approximately 50% to 70% of the glucose taken up by the placenta is converted into lactate, and the lactate produced then enters the fetal and maternal circulations at similar rates, under normal conditions (Sparks *et al.*, 1982). This baseline production of lactate by the placenta may not necessarily indicate anaerobic metabolism, but during significant

hypoxia, increments in lactate levels are likely to be due to fetal anaerobic metabolism (Milley, 1988). *In vitro*, lactate causes dilatation of the placental vessels and may play an important role in the events of fetal hypoxaemia which may occur during delivery (Omar *et al.*, 1993). Piquard and colleagues studied fetal and maternal lactate concentrations and the acid-base status of 589 women during labour and concluded that the increase in cord lactate in the second stage of labour was predominantly fetal in origin and only 6% of vaginal deliveries have a significant transfer of lactate from the woman to her infant (Piquard *et al.*, 1991).

The mean maternal venous lactate level found in the current study was at the upper end of our laboratory normal reference range (0.7 to 1.8 mmol/l) which was derived from the local non-pregnant population. Surprisingly, there is no lactate reference range published for the pregnant population. The mean maternal level found in the current study was higher compared to other studies where the maternal lactate levels were measured between 17 to 41 weeks gestation (Bon *et al.*, 2007). There could be a gestational effect on the lactate level as there are significant physiological changes in maternal circulation as the pregnancy advances.

Hypoglycaemia triggers the release of epinephrine which in turn causes the release of lactate from muscle (Ganong, 1995b). The average duration of fasting for women in the current study was 13.46 hours and all women had a blood glucose level within the normal range. Univariate analysis did not show an association between maternal or umbilical cord lactate levels and

the duration of last meal until delivery of infants. There is limited evidence in the literature on the effect of short period of fasting on the metabolic fuels of pregnant women. Metzger and colleagues studied the effect of fasting on the metabolic fuels of pregnant and non-pregnant women and concluded that after 12 to 14 hours of fasting, the level of glucose was lower in the pregnant group compared to the non-pregnant group, but both groups had similar levels of alanine, FFA and 3-hydroxybutyrate (Metzger *et al.*, 1982). After 16 hours of fasting, there were significantly greater rises in plasma FFA and 3-hydroxybutyrate levels, and significantly greater falls in plasma glucose and alanine levels in the pregnant women compared to non-pregnant women. This exaggerated response to overnight fasting, with a more pronounced decrease in plasma glucose and amino acids levels, and a greater rise in plasma FFA and ketone bodies, is termed 'accelerated starvation'. Schraag and colleagues also reported similar levels of FFA and 3-hydroxybutyrate in pregnant and non-pregnant women after 12 hours of fasting (Schraag *et al.*, 2007). Lactate levels were not measured in Metzger's study but with unchanged levels of alanine, FFA and 3-hydroxybutyrate in the pregnant women after 12 to 14 hours of fasting, this is likely the same for lactate level. A randomised controlled study assessing the risk and benefits of eating in labour has shown that lactate levels were similar in the eating and the starved group after 11.8 and 10.68 hours of labour respectively (Scrutton *et al.*, 1999). Hence it does not appear that short periods of fasting are a likely explanation for the relatively high lactate levels in the mothers in the current study.

Maternal samples were obtained from the foot vein immediately after delivery of the infant. The reason for taking the blood sample from a foot vein was simply to reduce any discomfort to the mother, as the majority had spinal anaesthesia for the delivery. It is not known whether the venous stasis caused by the spinal anaesthesia would have any effect on the lactate levels, but it is possible. Further studies will be required to examine this effect.

In the current study, umbilical cord lactate levels were also found to be significantly positively associated with arterial $p\text{CO}_2$ but negatively associated with BE. This is not surprising as lactate is the main contributor to the increase of BE in anaerobic metabolism. Krüger and colleagues have demonstrated a close correlation between lactate and both pH and BE (Krüger *et al.*, 1998). BE shows a linear correlation of the degree and duration of metabolic acidosis and is adjusted for the variation in $p\text{CO}_2$ (Ross and Gala, 2002).

In the current study, umbilical venous cord lactate was significantly and positively associated with maternal plasma glucose levels. Under normal conditions, there is a steady relationship between lactate and pyruvate with a ratio of about ten to one (Nordström, 2004). Hence, any condition that leads to an increase in glucose levels will increase pyruvate as well as lactate levels. Studies have shown that giving women a high dose of glucose (more than 25 gram of dextrose) prior to delivery could result in fetal hyperglycaemia and hyperinsulinaemia which in turn could lead to an

increase in lactate production and acidaemia (Kenepp *et al.*, 1982, Phillipson *et al.*, 1987).

Administration of β -mimetic drugs such as terbutalin to the mother has been shown to increase maternal and fetal blood glucose levels by accelerating glycogenolysis and increasing the release of insulin and glucagon (Westgren *et al.*, 1982). With regards to the relationship between umbilical venous cord lactate and catecholamine levels, a positive and significant correlation between umbilical venous cord lactate and NE levels were found in the current study. These findings concord with the study carried out by Nordström and colleagues who looked at the relationship of cord lactate levels to pH and catecholamines in 60 consecutive spontaneous vaginal deliveries (Nordström *et al.*, 1996). They showed a positive and significant association between lactate and EPI, NE and DA levels in the umbilical arterial blood. Lactate is the predominant gluconeogenic substrate of EPI-stimulated gluconeogenesis in both the liver and kidney (Meyer *et al.*, 2003). However in another study, the results suggested that fetal lactate production in the second stage of labour was mainly due to fetal anaerobic metabolism, secondary to hypoxia rather than as a consequence of catecholamine surge and increased hepatic glucose release (Nordström *et al.*, 2001).

Umbilical cord lactate levels were not associated with insulin levels but found to be significantly and negatively associated with glucagon levels in the current study. In adults, glucagon infusion or a test dose is associated with increased hepatic glucose production from both glycogenolysis and

gluconeogenesis (Søvik *et al.*, 1981). G-6-Pase is the rate limiting enzyme for pathways of glycogenolysis and gluconeogenesis (Ganong, 1995a) and is expressed at low levels in the human fetus *in-utero* and only increases in the postnatal period in term human infants (Burchell *et al.*, 1990). Hepatic glycogen accumulates in the human fetus in the later third trimester but the level falls considerably soon after birth (Shelley and Neligan, 1966). The glucagon stimulation test has been used as a diagnostic test in adult and children with genetic deficiency of G-6-Pase, and shows a typically flattened glucose response and an exaggerated lactate response (Chen and Burchell, 1995). The human fetus during labour and delivery is deficient of G-6-Pase as the enzyme levels only increase post-delivery. If glucagon can mobilise hepatic glycogen in the human fetus, G-6-P may be converted to lactate. To have a mechanism where lactate is negatively associated with glucagon is potentially protective for the fetus, as lactic acidaemia is not worsened.

5.4.3 Amino acids and glucose homeostasis in infants

A normal reference range for plasma amino acids has been published for adults (Armstrong and Stave, 1973), infants aged from day seven to four months (Anderson *et al.*, 1977) and children from 0 to 18 years of age (Lepage *et al.*, 1997) but no reference ranges have been published for the umbilical cord venous or arterial levels. It is not appropriate to compare the umbilical cord values to the postnatal levels as Anderson and colleagues have reported for instance that arginine, histidine, leucine, lysine and threonine all decrease within hours of birth (Anderson *et al.*, 1977).

The current study did not show a significant association between umbilical cord amino acid and glucose levels. This observation is possibly because gluconeogenic precursor supply alone does not influence the glucose concentration in normal physiological conditions. A study carried out by Jenssen and colleagues demonstrated that in normal adults, infusion of the glucogenic substrate, lactate did not increase the hepatic glucose output and the plasma glucose concentration under normal conditions (Jenssen *et al.*, 1990). They suggested that this was due to hepatic auto-regulation that prevented the substrate-induced increase in gluconeogenesis. A similar observation was also found in malnourished and recovered children (Kerr *et al.*, 1978). However, in the prolonged fasted adult, when the gluconeogenic precursor supply is low, infusion of alanine resulted in a hyperglycaemic response (Felig *et al.*, 1969). In preterm infants, the administration of alanine does not increase plasma glucose levels, which may be due to hepatic enzyme immaturity or to the low secretion levels of glucoregulatory hormones involved in gluconeogenesis (van Kempen *et al.*, 2003). The lack of association between alanine and glucose in the current study supports this view. Therefore, gluconeogenesis not only depends on an adequate precursor supply but it also requires adequate activity of the enzymes involved in gluconeogenesis and glycogenolysis which are regulated by an integrated metabolic and hormonal response.

5.4.4 Glucagon, insulin and glucose homeostasis in infants

Insulin to glucagon molar ratios have been used previously as an index of the endocrine pancreatic regulation on hepatic glucose metabolism (Unger,

1972). High molar ratios of insulin to glucagon favour anabolism whereas a low molar ratio favours catabolism. At term, the plasma insulin to glucagon ratio is high in the human fetus but falls rapidly and significantly, and then remains low after delivery, which in turn favours hepatic glucose release into the circulation through the processes of glycogenolysis and gluconeogenesis (Ktorza *et al.*, 1985). This current study has also shown a similar finding of a high insulin to glucagon ratio in the umbilical venous plasma. It is surprising to note that the glucagon levels were high in the umbilical venous cord given that the insulin levels were high too, but this may reflect the position that was described by Hume and colleagues (Hume *et al.*, 1999) as well as Jackson and colleagues (Jackson *et al.*, 2004) where it was concluded that glucagon did not play an important role in the neonatal glucose regulation.

The current study did not show an association between umbilical venous cord glucose and glucagon level. This could be due to the fact that the study population was in a 'stress free' environment during delivery. The response of fetal glucagon secretion to hypoglycaemia depends on the duration of hypoglycaemia. Acute hypoglycaemia does not influence glucagon secretion in the fetal mouse (Lernmark and Wenngren, 1972) nor in the human fetus (Schaeffer *et al.*, 1973). However, prolonged maternal fasting appears to increase glucagon secretion in fetal rats (Girard *et al.*, 1977) and also in fetal sheep (Schreiner *et al.*, 1981). In human adults, glucagon and epinephrine play an important role in increasing glucose levels during acute (Cryer *et al.*, 1984) as well as prolonged hypoglycaemia (Bolli *et al.*, 1984). Glucagon increases glucose production from the liver through stimulating

gluconeogenesis and glycogenolysis (Ganong, 1995b). However, in infants with severe and persistent hypoglycaemia after a controlled fast, cortisol and epinephrine levels were elevated but not glucagon levels (Hume *et al.*, 1999; Jackson *et al.*, 2004).

The current study also did not show an association between umbilical venous cord glucose and insulin levels. This may be because glucose metabolism in the human fetus is relatively unresponsive to insulin (Menon and Sperling, 1996). Studies on the effects of hyperinsulinaemia on glucose utilisation in fetal lambs has demonstrated that infusion of insulin results in a nearly twofold increase in glucose utilisation (Hay and Mezmarich 1986), but in the adult humans, this will produce a fivefold increase in glucose utilisation (Menon and Sperling, 1996).

There is no normal insulin reference range published for umbilical arterial or venous levels at birth or in children. The mean umbilical venous cord insulin level found in the current study was comparable to that reported by Wang and colleagues of 10.4 mU/l (Wang *et al.*, 1995). In the term human fetus, the plasma concentration of insulin (Kaplan, 1984) as well as the number and affinity of insulin receptors on monocytes (Thorsson and Hintz, 1977) are higher than in the adult. In contrast to the human adult, fetal insulin receptors are not down-regulated by hyperinsulinaemia (Kaplan, 1984). Insulin may be acting as the major growth driver *in-utero*; it is one of the key growth promoting hormones in the fetus acting directly through changes in fetal metabolism and indirectly through production of insulin-like growth

factors (IGF) (Fowden, 1995). Long term infusions of insulin in fetal rhesus monkeys resulted in increased fetal growth and eventually fetal macrosomia (Susa *et al.*, 1984). In diabetic pregnant women, hyperglycaemia can lead to an increase in insulin secretion and beta cell hyperplasia in the fetus, which can result in a macrosomic infant (Nelson-Piercy, 2002). Such infants are at risk of hypoglycaemia due to hyperinsulinaemia in the early neonatal period. Insulin increases glucose uptake in insulin-sensitive tissue and promotes glyconeogenesis, as well as lipid synthesis; whereas human GH antagonises the effects of insulin on carbohydrate and lipid metabolisms (Ganong, 1995b). Therefore, increased GH secretion may be associated with insulin resistance initially, but eventually hypersecretion of insulin occurs to counterbalance the insulin resistance (Menon and Sperling, 1996). During puberty, there is decreased sensitivity to insulin (Bloch *et al.*, 1987) and high levels of hGH found in children (Rose *et al.*, 1991). Similar changes may also occur in the fetus towards the end of a pregnancy when the fetus gains most weight (Gilstrap, 1999b).

5.4.5 Cortisol and glucose homeostasis in infants

There are limited reference data for plasma cortisol levels at birth reported in the literature. A recent study by Garagorri and colleagues reported reference values for plasma adrenal steroid levels, from birth until six months of age, generated from 138 term well infants delivered by spontaneous vaginal delivery (Garagorri *et al.*, 2008). They showed that the level of cortisol remains unchanged from birth until six months of age (Garagorri *et al.*, 2008). The mean umbilical venous cortisol level of 139 nmol/l found in the

current study was lower than the reported value of 490 nmol/l by Garagorri and colleagues (Garagorri *et al.*, 2008). This is not surprising as several studies have shown that umbilical cortisol levels were significantly lower in infants delivered by elective caesarean section than those delivered vaginally (Lao and Panesar, 1989; Mears *et al.*, 2004; Miller *et al.*, 2005). However, there appears to be a big variation in the mean umbilical venous cord cortisol levels in infants delivered by elective caesarean section reported in the literature (Gitau *et al.*, 2001; Vogl *et al.*, 2006; Zanardo *et al.*, 2006). The mean umbilical venous cord cortisol level found in the current study was lower than that reported by Vogl and colleagues of 225 nmol/l (Vogl *et al.*, 2006) as well as Gitau and colleagues of 347 nmol/l (Gitau *et al.*, 2001) but higher than that reported by Zanardo and colleagues of 125 nmol/l (Zanardo *et al.*, 2006). However, all these studies were small studies with only 12 to 29 samples and hence the results may not be valid.

This current study did not find an association between umbilical venous cord glucose and cortisol levels. This could be due to the fact that during intrauterine life, the maintenance of glucose levels in the human fetus is dependent upon the maternal supply of glucose and there is no significant production of glucose by the fetus *in-utero* (Kalhan *et al.*, 1979). Maternal cortisol levels were not measured in this current study but one would expect that the concentrations of cortisol levels in the mother are appreciably higher than that of the fetus (Gitau *et al.*, 1998). The placenta exhibits high levels of the enzyme 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD-2) in the syncytiotrophoblastic cells, which converts cortisol to metabolically inert

cortisone and thus, protects the fetus from the high maternal cortisol concentrations (Seckl *et al.*, 1995). Gitau and colleagues also demonstrated that there was a linear relationship between fetal and maternal cortisol concentrations, and that the levels were not related to gestational age (Gitau *et al.*, 1998). A study of the conversion of maternal cortisol to cortisone during placental transfer to the human fetus at mid-gestation showed that 15% of ^3H -cortisol crossed the placenta unmetabolised (Murphy *et al.*, 1974). Despite the presence of 11β -HSD-2, a functional barrier to restrict the transfer of cortisol from the mother to the fetus, there is still approximately 10% to 20% of maternal cortisol transfer across the placenta to the fetus (Gitau *et al.*, 1998). Therefore, any condition that increases maternal cortisol levels could lead to an increase in the fetal cortisol levels during pregnancy.

5.4.6 Catechols and glucose homeostasis in infants

The current study demonstrated high levels of catecholamines in the umbilical venous plasma which is in keeping with the results of other studies (Lagercrantz and Bistoletti, 1977; Eliot *et al.*, 1980). As discussed before (see pages 25 to 27), this postnatal surge is important for the neonatal cardiovascular, respiratory and metabolic adaptation at birth (Sperling *et al.*, 1984).

Studies in fetal sheep showed that the placenta is responsible for the clearance of a large fraction of circulating catecholamines (Bzoskie *et al.*, 1995). Eisenhofer concluded that as much as 50% of the circulating catecholamines in the fetus are cleared by the placenta (Eisenhofer, 2001).

MAO-A activity in the placenta is high, second only to the liver, and localized predominantly to the syncytiotrophoblastic layer (Billett, 2004). In the placenta, NET supports both the uptake of NE and DA (Ramamoorthy *et al.*, 1993). Bzorkie and colleagues have shown an inverse relationship between placenta NET mRNA expression and umbilical cord NE levels (Bzorkie *et al.*, 1995). Trophoblastic cells invade the media of maternal spiral arteries and these cells express high levels of NET and VMAT2 mRNA so may prevent maternal plasma NE from contracting the uterine vessels and hence maintain circulatory homeostasis (Bottalico *et al.*, 2004). NET mRNA is also expressed in the syncytiotrophoblast cells of the chorionic villi of the placenta and expression in such cells may function to reduce the fetal NE plasma levels (Bottalico *et al.*, 2004). High plasma levels of NE were found in pregnancies complicated with pre-eclampsia (Manyonda *et al.*, 1998; Kaaja *et al.*, 1999). Expression of NET and EMT in the placenta from pre-eclamptic pregnancies is reduced, which supports the concept that placenta blood flow may be compromised through inappropriate vascular exposure to higher catecholamines as the expression of transporters is reduced (Bottalico *et al.*, 2004). Several studies have also shown that the expression and activity of MAO-A is reduced in the placentae from pre-eclamptic pregnancies (Carrasco *et al.*, 2000, Sivasubramaniam *et al.*, 2002) consistent with reduced NET expression and higher maternal NE levels.

Fetal membranes have also been found to be responsible for catecholamine metabolism. MAO, COMT, and PNMT have been shown to be present in human fetal membranes at term, with MAO activity particularly high in

chorion compared to amnion (Casimiri *et al.*, 1991). Hence, at birth, the increase in circulating catecholamine levels is due partly to the significant decrease in clearance rates, secondary to the removal of the placenta (Stein *et al.*, 1993) as well as the fetal membranes.

The limited human data available suggest that levels of the sulfated catechols (EPI sulfate, NE sulfate, DHPG sulfate, DOPA sulfate, DOPAC sulfate) were lower in umbilical venous plasma than in venous plasma of children and adults (Ratge *et al.*, 1986; Eichler *et al.*, 1989). As mentioned earlier (see page 33), *SULT1A3* is expressed at high levels in the fetal liver but decreases significantly in the late fetal-early neonatal period (Richard *et al.*, 2001). Richard and colleagues speculated that prior to delivery the active hepatic sulfoconjugation would cease to allow free catechols to rise for adaptation to extrauterine life to take place (Richard *et al.*, 2001). The results of this current study, where there are low levels of sulfated catechols in the umbilical venous plasma, would be in keeping with this speculation. The combined effects of the cessation of hepatic sulfoconjugation as well as the decreased clearance from the placenta and fetal membrane secondary to disconnection allow the free catecholamine levels to rise, an essential prerequisite for adaptation to extrauterine life.

Measurement of catechols in the umbilical venous cord in the current study showed a marked predominance of NE, as much as ten times over EPI levels in these term infants. This result is similar to other studies (Falconer and Lake, 1982; Vogl *et al.*, 2006). Adults and children between 5 to 17

years old also showed a similar pattern of higher plasma NE levels compared to EPI levels under resting conditions (Weise *et al.*, 2002). However, this is in contrast to the study carried out by Ekblad and colleagues on preterm infants where the ratio of NE to EPI was reversed; preterm infants were found to have a higher level of EPI than NE on day two (Ekblad *et al.*, 1992). Postnatal changes in EPI and NE levels in infants are more problematic to interpret as they may be related to differences in degrees of prematurity and mode of delivery.

CHAPTER 6

DISCUSSION

Preterm infants are at high risk of adverse neonatal outcome including brain damage, chronic lung disease, retinopathy of prematurity, necrotizing enterocolitis, infection, behavioural problems, and neurodevelopmental delay. There are many factors, which can result in brain damage in this population; one such factor is hypoglycaemia. In preterm infants, failure to up-regulate G-6Pase, the key enzyme of hepatic gluconeogenesis, in the immediate postnatal period is common. This may result in defective glucose homeostasis. Previous work by our group has shown that up to 18% of preterm infants at the time of discharge home have low blood glucose levels leaving them vulnerable to repeated hypoglycaemia and risk of brain damage (Hume *et al.*, 1999). Normally the hepatic control of glucose production switches on at the time of birth but this appears to fail in some preterm infants. These failures to switch on the key gluconeogenic genes are not confined to preterm infants but also to some term infants. In 1989, Burchell and colleagues described a small cohort of eight term cases of sudden infant death syndrome (SIDS) and found that these infants had low G-6-PC1 activity and expression (Burchell *et al.*, 1989b). The proportion of term infants who have delayed onset of expression of G-6-Pase is not known. The reasons for failure to switch on in some infants is not known. Since the mid 1990s, investigators taken two distinct paths to further investigate this delay in expression of hepatic G-6-PC1.

Firstly, a genetic approach particularly of term and preterm infants who had sudden and unexpected deaths. Forsyth and colleagues confirmed that G-6-PC1 activity is low in term SIDS infants as well as preterm SIDS and non-SIDS infants in a large cohort of such infants (Forsyth *et al.*, 2007). The study also showed that a functional polymorphism in the promoter region of G-6-PC1 was associated with reduced expression of the protein and reduced hepatic enzyme activity and that this was more common in infants who died of SIDS (Forsyth *et al.*, 2007).

Secondly, through understanding the regulation and hormonal control mechanism in the switching on of perinatal genes, it was postulated that there may be defects in such mechanisms in some infants. Hormonal dysfunctions have now been described in preterm infants including hypothalamic-pituitary-thyroid insufficiencies at birth (Murphy *et al.*, 2004), transient hypothyroxinaemia (Frank *et al.*, 1996), glucagon unresponsiveness and insulin resistance (Jackson *et al.*, 2003). It is highly probable that a combination of hormonal dysfunctions are present at the time of birth especially in preterm infants but these need to be assessed against a background of normal adaptation of the term infant.

At present there is little information about the normal hormonal profiles in term and preterm infants around the time of delivery. The conventional concepts of glucose homeostasis and regulation during labour and the early postnatal period in humans are extrapolated mainly from animal studies under controlled laboratory conditions. Studies that have been done in

humans have been limited to circumscribed areas of investigation of particular hormones or metabolites, which can create a very misleading picture. Hence the studies undertaken for this thesis were carried out to inform the investigation of hormonal and metabolic control, and to establish “normal” ranges for infants delivered by elective caesarean section. Three pilots studies were carried out to clarify the methodology to enable the main study to be undertaken. These preliminary studies emphasised the importance of prompt sampling of cord blood and subsequent analysis of samples, as some hormones and metabolites such as glucose, lactate and catechols, change significantly with post-delivery time.

The main study (described in Chapter 5) is the largest series of data combining assays of representative alternative metabolic substrates for brain metabolism (glucose, lactate, 3-hydroxybutyrate, FFA and amino acids), the regulatory hormone insulin, and those counter-regulatory to insulin (glucagon, cortisol, hGH and catechols) in term infants at birth. Only three infants were found to have an umbilical blood glucose level of less than 2.6 mmol/l at birth and this was entirely expected as the study population was in a ‘stress-free’ environment during delivery. However the effect of ‘stress’, for example from vaginal delivery or emergency caesarean section, may increase the number of hypoglycaemic infants at birth. If this scenario is correct it is crucial (for protection of brain integrity) that infants are able to use alternative substrate(s) in the event of low blood glucose levels. Overt hypoglycaemia at delivery has been described in infants (Burchell *et al.*, 1989b) but more subtle presentations are likely to exist. The most vulnerable

infant is likely to be the one with a severe metabolic or hormonal defect or where compensatory mechanisms are defective or compromised. Further studies are clearly required in infants delivered vaginally, or by emergency operative deliveries, to define the range of metabolites and hormones in these circumstances, and to consider whether an observational study should be planned on blood glucose levels at birth in term infants in relation to long-term neurodevelopment outcomes.

The current study showed a significant and positive association between maternal and fetal glucose levels, but no association of umbilical venous cord glucose with insulin, glucagon, hGH, cortisol, amino acids and catechols levels. High levels of insulin and catechols were noted at birth. The majority of the infants at delivery showed normal glucose levels despite higher levels of insulin. Insulin appears to act as the major growth driver *in-utero*, and this state of hyperinsulinaemia is countered by a positive maternal-placental glucose gradient as well as higher fetal glucagon and hGH levels which maintain normoglycaemia. Catecholamines play an important role in intrauterine life adaptation but may also play an important role in triggering the transition from maternal dependence to independent postnatal glucose homeostasis. The evidence to support this hypothesis was not provided by the current study and will need to be evaluated in a larger study with a larger proportion of infants with a wider range of blood glucose levels, particularly where these are low and in the conventional range for hypoglycaemia. There is evidence to show that there is an arterio-venous difference in EPI and NE in cord infant blood with venous levels lower than arterial (Eliot *et al.*,

1980, Falconer and Lake, 1982). Postnatal levels of EPI and NE are high (e.g. Eliot *et al.*, 1980) consistent with high amounts of VMA and HVA in postnatal urine (Gabriel *et al.*, 1983). These observations suggest that there is a feto-placental-maternal metabolism or transport of catechols. This thesis describes for the first time a full range of catechols and their sulfated conjugates levels in non-stressed term infants; this baseline information can now be used to research a variety of perinatal scenarios, including establishing the inter-relationship between fetal and maternal metabolism of catechols.

The main strength of this work is that a whole of range of analytes were measured in one infant as opposed to many other studies which only measured one or two analytes which could create a misleading picture. At the same time, this was also the limitation of the study as the range of analytes measured necessitated the use of venous blood samples as opposed to arterial cord blood samples, which would have provided a more accurate reflection of the fetal metabolism. Our unpublished data on the catechols levels from 60 paired arterial and venous samples from infants who delivered by elective caesarean section showed that the mean catechol levels were significantly higher in the umbilical artery compared to the umbilical vein samples. The sulfated conjugates levels were also significantly higher in the umbilical artery compared to the umbilical vein samples, with the exception of DOPA sulfate. Studies have shown that there are no correlations between maternal and fetal catecholamine levels (Gülmezoglu *et al.*, 1996) and that the fetus is able to mount a sympatho-

adrenal response independent from the mother (Giannakouloupoulos *et al.*, 1999). As discussed in the previous chapter, the placenta plays an essential role in catecholamine homeostasis in the fetus and as much as 50% of the circulating catecholamines are cleared by the placenta (Eisenhofer, 2001). The placenta has high MAO-A activities (Billet, 2004) and SULT1AS expression (Stanley *et al.*, 2001).

A small study carried out by Scheepers and colleagues showed no differences between umbilical arterial and venous levels of glucose, FFA, lactate and beta-hydroxybutyrate (Scheepers *et al.*, 2004). Further studies are clearly required to establish the arterio-venous difference of various hormones and metabolites to determine whether the venous and arterial levels are comparable as well as to investigate the role of placenta metabolism and feto-maternal transfer.

In conclusion, the importance of this dataset has been the provision of normal values which can inform management of term infants and provide comparative data for future research projects. This extensive data set has produced a baseline data in which reference values can be generated and will be valuable in evaluating any effects of antenatal or intrapartum factors on the hormonal and metabolic profiles of infants at birth.

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APPENDICES

Table 5.6: Univariate analysis model for cord glucose and lactate

Step 1	Outcome dependent	Explanatory variables
Bivariate analysis	Cord glucose Cord lactate	Categorical variables Maternal smokers Antenatal events Antepartum haemorrhage Asthma Epilepsy Thyroid disease Diabetes Hyperemesis gravidarum <i>Streptococcus agalactia</i> carriage Tractocile treatment Betamethasone injection Hypertension Treated UTI Treated vaginal discharge Reason for C/S Breech presentation Maternal request Other reasons# Previous caesarean section Ephedrine received Phenylephrine received Atropine received Glycopyrrolate received SCBU admission Infant sex Neonatal resuscitation Continuous variables Gestational age Maternal age Maternal BMI at delivery Placenta weight Apgar score at 1 min Apgar score at 5 min Cord blood arterial pH Cord blood arterial BE Cord blood arterial pO ₂ Cord blood arterial pCO ₂ Cord blood arterial cHCO ₃ Infant birth weight Infant birth weight ratio Infant OFC Infant birth length Maternal glucose Maternal lactate Time interval from maternal fasting to cord sampling Cord insulin Cord glucagon Cord hGH Cord cortisol Cord EPI

Table 5.6: continuation from above table

Step 1	Outcome dependent	Explanatory variables
		Continuous variables
		Cord EPI sulfate
		Cord NE
		Cord NE sulfate
		Cord DOPA
		Cord DOPA sulfate
		Cord DHPG
		Cord DHPG sulfate
		Cord DOPAC
		Cord DOPAC sulfate
		Cord 3-hydroxybutyrate
		Cord free fatty acids
		Cord amino acids
		Alanine
		Aminobutyric acid
		Arginine
		Citrulline
		Cystine
		GABA
		Glutamic acid
		Glutamine
		Glycine
		Histidine
		Isoleucine
		Leucine
		Lysine
		Methionine
		Ornithine
		Phenylalanine
		Serine
		Taurine
		Threonine
		Tyrosine
		Valine

UTI urinary tract infection, **C/S** caesarean section, **SCBU** special care baby unit, **BMI** body mass index, **BE** base excess, **cHCO₃** bicarbonate, **OFC** occipital-frontal head circumference, **hGH** human growth hormone, **EPI** epinephrine, **NE** norepinephrine, **DOPA** dihydroxyphenylalanine, **DHPG** dihydroxyphenylglycol, **DOPAC** dihydroxyphenylacetic acid

#Other reasons included cephalopelvic disproportion, Crohn's disease, bowel problems as a result of a previous delivery, and an unstable lie

Table 5.7: Univariate regression model for cord glucose and lactate

Step 2	Outcome dependent	Explanatory variables
Univariate regression analysis	Cord glucose	Significant factors from step 1 (Table 4.6)
		Ephedrine received Neonatal resuscitation required Reasons for caesarean section Maternal glucose Cord EPI Cord EPI sulfate Cord NE Cord 3-hydroxybutyrate Cord alanine Cord lysine
		Important factors identified from literature Maternal diabetes Cord insulin Cord glucagon Cord hGH Cord cortisol
	Cord lactate	Significant factors from step 1 (Table 4.6)
		Apgar 5 min Arterial BE Maternal glucose Maternal Lactate Cord EPI Cord EPI sulfate Cord NE Cord NE sulfate Cord DOPA Cord DOPA sulfate Cord DHPG Cord DHPG sulfate Cord alanine Cord leucine Cord tyrosine Cord isoleucine Cord valine
		Important factors identified from literature Cord glucose Cord insulin Cord glucagon Cord hGH Cord cortisol

EPI epinephrine, **NE** norepinephrine, **hGH** human growth hormone, **BE** base excess, **DOPA** dihydroxyphenylalanine, **DHPG** dihydroxyphenylglycol,

GLUCOSE AND LACTATE LEVELS IN CORD BLOOD SAMPLES ROUTINELY COLLECTED AT DELIVERY FOR ESTIMATION OF BLOOD GAS AND ACID-BASE STATUS IN TERM INFANTS

(Reference number 033/04)

We invite you to participate in a research project. We believe it to be of potential importance. However, before you decide whether or not you wish to participate, we need to be sure that you understand firstly why we are doing it, and secondly what it would involve if you agreed. We are therefore providing you with the following information. Read it carefully and be sure to ask any questions you have, and, if you want, discuss it with outsiders. We will do our best to explain and to provide any further information you may ask for now or later. You do not have to make an immediate decision.

THE BACKGROUND TO THE STUDY

Every year, there are a few babies who suffer from brain damage at delivery. There are a number of possible causes. We suspect that low blood glucose (sugar) level at birth is one of the possible causes. We would like to study this further in healthy term infants. If this study shows low blood glucose (sugar) level may be a problem then we will seek a solution to this in the future.

We would like to ask mothers who deliver their infants at term to participate in this initial study.

The University of Dundee pays the salaries of the researchers. We will seek further funds from charities to pay for the blood tests.

WHAT DOES THE STUDY ENTAIL?

We routinely take blood from the umbilical cord after the baby is delivered to measure the oxygen level. This is done for all deliveries. For this study, an additional small sample of blood will also be retained (about a teaspoonful). It does not involve taking blood from you or your baby. We will use this blood to measure the glucose levels and other related substances, which are used for energy, as well as hormones which regulate glucose levels. We shall also abstract from your medical notes information about your health during pregnancy, your progress in labour and about your baby at birth.

WHAT ARE THE DISCOMFORTS, RISKS AND SIDE EFFECTS?

There are no risks to your baby as the blood sample will be taken from the umbilical cord after delivery of the placenta.

WHAT WILL HAPPEN TO THE INFORMATION COLLECTED IN THE STUDY?

All aspects of the study concerning you and your baby will remain confidential. Only the project researchers will have access to the information collected in the study. The information about you will be stored in a locked computer file accessed by password. Your name will not be entered in this file, only a unique record number. Only a limited number of researchers in Dundee will know the names of the participants. A floppy disc containing unique record numbers and names will be kept in a locked filing cabinet.

This is a study to find out what are the normal levels of glucose and other related substances in cord blood at the time of delivery. It is possible we may find babies who have higher or lower levels than other babies. If we think this may affect the future health of your baby, we will discuss this with you. You will be offered an appointment with Professor Robert Hume Consultant Paediatrician at Ninewells to discuss this further. We will not tell your GP that you are taking part in this study but if there is concern about your baby's health we will involve your GP. If your baby's results are similar to other babies in the study we will not routinely tell you the results.

WHAT ARE MY RIGHTS?

Participation in this study is entirely voluntary and you are free to refuse to take part or to withdraw from the study at any time without having to give a reason and without this affecting your future medical care or your relationship with medical staff looking after you.

You are free to discuss the study and seek advice about participation from others. There will be no payment for participation.

We believe it is safe for you to take part in this study but you are insured through NHS Tayside should something go wrong and compensation is needed.

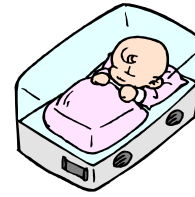
The Tayside Committee on Medical Research Ethics, which has responsibility for scrutinising all proposals for medical research on humans in Tayside, has examined the proposal and has raised no objections from the point of view of medical ethics. It is a requirement that your records in this research, together with any relevant medical records, be made available for scrutiny by monitors from NHS Tayside and the Regulatory Authorities. But should you not wish this you can refuse and this information will be recorded on your consent form.

A copy of this information sheet and consent form will be given to you to keep. Should you require more information or have any questions, you could contact Dr. Daisy Koh, the main co-ordinator of this study at Ninewells Hospital on 01382 660111 Bleep 4165.

SER No

office use only

A study of glucose and lactate levels on cord blood



BACKGROUND INFORMATION

- 1 Mother's surname _____
- 2 Mother's DoB _____
dd mm yyyy
3. Mother's CHI or ID number
- 5 Gestation at delivery completed weeks
- 6 Current smoker ₁ Non-smoker ₂ Ex-smoker ₃
- 7a Booking Weight (Kg) • 8 Booking height (m) •

MATERNAL MEDICAL HISTORY

- 9 ANTEPARTUM HAEMORRHAGE^g Yes ₁ No ₂
- if yes, episode 1 at gestation weeks
 episode 2 at gestation weeks
 episode 3 at gestation weeks

what was the cause?

Tick if yes

- abruption ₁
- placenta praevia ₂
- vasa praevia ₃
- other ₄ please specify _____
- Not known ₅

- 10 ASTHMA^g Yes ₁ No ₂ specify treatment _____
- 11 EPILEPSY^g Yes ₁ No ₂ specify treatment _____

- 12 THYROID DISEASE concurrent with pregnancy Yes ₁ No ₂

^g Antepartum haemorrhage is vaginal bleeding after 24 weeks

Asthma and epilepsy should be considered present if the women is on current treatment

Tick all that apply

- a if yes,**
- hyperthyroid ☐₁
- hypothyroid ☐₁
- simple non-toxic goitre ☐₁
- other thyroid enlargement ☐₁
- auto immune thyroiditis ☐₁
- Other, please specify ☐₁

- b** Current T4 treatment Yes ☐₁ No ☐₂ **if yes,** T4 mg/day/Last menstrual period
T4 mg/day/delivery

- c** Family history thyroid disease Yes ☐₁ No ☐₂ **if yes,** Mother ☐₁
Father ☐₁
Siblings ☐₁ out of

- d** Hyperthyroid treatment Yes ☐₁ No ☐₂
if yes:
- | | Regimen prior
To pregnancy | Regimen during
pregnancy |
|--------------------|---------------------------------------|---------------------------------------|
| Carbimazole | <input type="checkbox"/> ₁ | <input type="checkbox"/> ₁ |
| Surgery | <input type="checkbox"/> ₁ | <input type="checkbox"/> ₁ |
| Thiouracil | <input type="checkbox"/> ₁ | <input type="checkbox"/> ₁ |
| radioactive iodine | <input type="checkbox"/> ₁ | <input type="checkbox"/> ₁ |

- 13 DIABETES MELLITUS** Yes ☐₁ No ☐₂
- a if yes,** Insulin dependent diabetes mellitus ☐₁
or, Gestational diabetes: insulin required ☐₂
or Gestational diabetes: non-insulin ☐₃

- b** Age IDD diagnosed yrs
- c** IDD insulin at booking units/day
- d** IDD insulin at delivery units/day

- e** Gestational diabetes fasting blood glucose mmol/l

- f** Gestational diabetes 2 hour blood glucose mmol/l _____
after 75g OGTT

- g** Gestational diabetes diagnosis made at _____ completed weeks gestation

- h** Insulin for Gestational diabetes started at _____ completed weeks gestation

- i** Gestational diabetes max insulin dosage units/day Diet only ☐₁

- 14 HYPEREMESIS** Yes ☐₁ No ☐₂

if yes, *tick as many as apply* admitted ☐ 1
Ketonuria ☐ 1
IV fluids given ☐ 1

15 ANY OTHER CONDITIONS *please specify*

16 a DID THE MOTHER HAVE GROUP B STREPTOCOCCUS ☐ Yes ☐ No

If yes

b At what gestation was Group B identified weeks

c If yes was treatment *tick as appropriate* antenatal ☐₁ intrapartum ☐₂ post natal ☐₃

d Antibiotic *please specify*

MATERNAL MEDICATIONS

17 a Ritodrine ☐ Yes ☐ No

17aa Tractocile ☐ Yes ☐ No

b initial / / **c** final^g / / **d** total days^g treatment .

e total ritodrine dosage (mg)

f maternal weight start treatment (kg)

g Date & time final dose ____ / ____ / ____
dd mm yyyy 24 hour clock

18 a Nifedipine 1 2

b initial / / **c** final^g / / **d** total days^g treatment .

e total nifedipine dosage (mg)

f maternal weight start treatment (kg)

g Date & time final dose _____/_____/_____
dd mm yyyy 24 hour clock

19 a First Steroid Course Yes ☐ ₁ No ☐ ₂ if yes, Name of steroid _____

b initial / / **c** final^g / / **d** total steroid dosage (mg)

e maternal weight start treatment (kg)

f Date & time final dose _____/_____/_____
dd mm yyyy 24 hour clock

Yes No

^g **Final** date is the date is the final date of treatment or the date of delivery *whichever* is first

20 a **Second Steroid Course** ☐₁ ☐₂ if yes, Name of steroid _____

b initial ____/____/____ c final^g ____/____/____ d total steroid dosage (mg) _____
dd mm yyyy dd mm yyyy

e maternal weight start treatment (kg) .

f Date & time final dose ____/____/____ 24 hour clock
dd mm yyyy

IF MORE COURSES GIVEN, USE OPPOSITE PAGE

21 a **Indomethacin**

Yes No
☐₁ ☐₂

b initial ____/____/____ c final^g ____/____/____ d total indomethacin dosage (mg) _____
dd mm yyyy dd mm yyyy

e maternal weight start treatment (kg) .

f Date & time final dose ____/____/____ 24 hour clock
dd mm yyyy

MATERNAL HYPERTENSION during pregnancy^g

22 Is the mother hypertensive? Yes No ☐₁ ☐₂ → Go to Q27

23 a Max Systolic BP during pregnancy mmHg b Max Diastolic BP during pregnancy mmHg

c Gestation at max BP weeks

d Max urinary protein g/l

e Gestation at max urinary protein weeks

f Max plasma urate mmol/l

g Max plasma creatinine μmol/l

h Min blood platelets 10⁹/l

i Max plasma alanine aminotransferase u/l

ANTI HYPERTENSIVE DRUGS during pregnancy ONLY

NB final date is date finish treatment OR delivery WHICH EVER IS FIRST

24 a First drug please specify _____

b start ____/____/____
dd mm yyyy

c final ____/____/____
dd mm yyyy

25 a Second drug please specify _____

b start ____/____/____
dd mm yyyy

c final ____/____/____
dd mm yyyy

26 a Third drug please specify _____

b start ____/____/____
dd mm yyyy

c final ____/____/____
dd mm yyyy

MATERNAL ANTICONVULSANT DRUG during pregnancy ONLY

NB final date is date finish treatment OR delivery WHICH EVER IS FIRST

- 27 a Drug 1 *please specify* _____
 b start ____/____/____ dd mm yyyy c final ____/____/____ dd mm yyyy
- 28 a Drug 2 *please specify* _____
 b start ____/____/____ dd mm yyyy c final ____/____/____ dd mm yyyy
- 29 a Drug 3 *please specify* _____
 b start ____/____/____ dd mm yyyy c final ____/____/____ dd mm yyyy

ANY (ORAL OR IV) MATERNAL STEROIDS (eg for asthma) other than dexamet etc. for prevention of RDS.^g

Course 1

- 30 a Name _____
 b start^g ____/____/____ dd mm yyyy c finish^g ____/____/____ dd mm yyyy
 d total daily dosage _____ mg e total days treatment
 f maternal weight start treatment kg

Course 2

- 31 a Name _____
 b start^g ____/____/____ dd mm yyyy c finish^g ____/____/____ dd mm yyyy
 d total daily dosage _____ mg e total days treatment
 f maternal weight start treatment kg

Course 3

- 32 a Name _____
 b start^g ____/____/____ dd mm yyyy c finish^g ____/____/____ dd mm yyyy
 d total daily dosage _____ mg e total days treatment
 f maternal weight start treatment kg

TREATED MATERNAL GU INFECTIONS (*In the week prior to delivery*)^g

- a Urinary tract infection ☐₁ ^{Yes} ☐₂ ^{No} if yes,
 b Treatment (*specify*) _____ c Dosage _____
 d Organism (*specify*) _____ e Date ____/____/____

- 34 Yes No

^g **Finish date** is date finish treatment OR delivery WHICH EVER IS FIRST

Start date is the date of last menstrual period for mothers who were on steroids throughout pregnancy, OR, true start date as applicable

Treated maternal GU infections defined as treated and not simply culture positive

- a Vaginal discharge ☐₁ ☐₂ if yes,
 b Treatment (*specify*) _____ c Dosage _____
 d Organism (*specify*) _____ e Date ____/____/____

DELIVERY AND BIRTH DETAILS

- 35 a Was labour onset Spontaneous ☐₁ Induced ☐₂
 b if induced, how Artificial rupture membranes ☐₁
 Prostaglandins pessary ☐₂
 Syntocin ☐₃
 c reason(s) for induction *please specify* _____
- 36 a Was labour augmented? Yes ☐₁ No ☐₂
 b if yes was this by Syntocin Yes ☐₁ No ☐₂
 c reason(s) for augmentation *please specify* _____

37 Type of delivery**tick one box**

- a Spontaneous cephalic vaginal ☐₁ d Elective caesarean ☐₄
 b Vaginal breech ☐₂ e Emergency caesarean ☐₅
 c Instrumental cephalic vaginal ☐₃

38 Drugs received during labour**tick as many as apply**

- a None received ☐₁ d Epidural / spinal ☐₁
 b Entonox ☐₁ e Opiate ☐₁
 c General anaesthesia ☐₁ f Other, *please specify* ☐₁

39 a Duration of first stage labour ____ hrs ____ mins

40 How long were the membranes ruptured? ____ days ____ hrs ____ mins

41 Placental weight gm**DELIVERY OUTCOMES**

- 42a Date of birth ____/____/____ 42b Time of birth 24 hour clock
 43 Apgar score 1 minute 44 Apgar score 5 minutes
 47 Resuscitation required Yes ☐₁ No ☐₂
 if yes, Narcan and/or facial O₂ ☐₁ IPPV by tube ☐₃
 IPPV by mask ☐₂ IPPV and drugs ☐₄

INFANT CHARACTERISTICS

48 Infant's name

First _____ Surname _____

50 Infant's sex Male ☐₁ Female ☐₂**52 Infant's CHI or ID number****54 Birth length** • cm**49 Infant's DoB**____/____/____
dd mm yyyy**51 Multiple birth?** Yes ☐₁ No ☐₂
if yes, twin ☐₁ Triplet ☐₂ Other ☐₃**53 Infant's birth weight** gm**55 Birth OFC** • cm**BIOCHEMISTRY: INFANT****AT BIRTH****45 Umbilical artery pH** **83 Umbilical vein pH** **46b Umbilical artery pCO₂** kPa**83a Umbilical vein pCO₂** kPa**46c Umbilical artery pO₂** kPa**83b Umbilical vein pO₂** kPa**46d Umbilical artery cHCO₃** mmol/l**83c Umbilical vein cHCO₃** mmol/l**46a Umbilical artery BE** mmol/l**83d Umbilical vein BE** mmol/l**84 Glucose level** mmol/l**85 Lactate level** mmol/l**Name of person who completed the abstraction form** _____**Position** _____**Date completed** ____/____/____

Additional steroid courses

Third Steroid Course

Yes No

a ☐₁ ☐₂ if yes, Name of steroid _____

b initial ____/____/____ c final^g ____/____/____ d total steroid dosage (mg) _____
dd mm yyyy dd mm yyyy

e maternal weight start treatment (kg) .

f Date & time final dose ____/____/____ 24 hour clock
dd mm yyyy

Fourth Steroid Course

a

Yes No	<input type="checkbox"/> ₁	<input type="checkbox"/> ₂	if yes, Name of steroid _____
-----------	---------------------------------------	---------------------------------------	--------------------------------------

b initial ____ / ____ / ____ **c** final⁹ ____ / ____ / ____ **d** total steroid dosage (mg) _____

dd mm yyyy dd mm yyyy

e maternal weight start treatment (kg) •

f Date & time final dose ____ / ____ / ____

dd mm yyyy 24 hour clock

Fifth Steroid Course

a

Yes <input type="checkbox"/>	No <input type="checkbox"/>	if yes, Name of steroid _____
<input type="checkbox"/> ₁	<input type="checkbox"/> ₂	

b initial ____ / ____ / ____ **c** final^g ____ / ____ / ____ **d** total steroid dosage (mg) _____

dd mm yyyy dd mm yyyy

e maternal weight start treatment (kg) .

f Date & time final dose ____ / ____ / ____ 24 hour clock

dd mm yyyy

MEASURING CHANGES IN VENOUS CORD BLOOD GLUCOSE, LACTATE, BLOOD GASES AND ACID-BASE VALUES WITH TIME FOLLOWING DELIVERY

(Reference number 033/04)

We invite you to participate in a research project. We believe it to be of potential importance. However, before you decide whether or not you wish to participate, we need to be sure that you understand firstly why we are doing it, and secondly what it would involve if you agreed. We are therefore providing you with the following information. Read it carefully and be sure to ask any questions you have, and, if you want, discuss it with outsiders. We will do our best to explain and to provide any further information you may ask for now or later. You do not have to make an immediate decision.

THE BACKGROUND TO THE STUDY

Every year, there are a few babies who suffer from brain damage at delivery. There are a number of possible causes. We suspect that low blood glucose (sugar) level at birth is one of the possible causes. We would like to study this further in healthy term infants. If this study shows low blood glucose (sugar) level may be a problem then we will seek a solution to this in the future.

We would like to ask mothers who deliver their infants at term to participate in this initial study.

The University of Dundee pays the salaries of the researchers. We will seek further funds from charities to pay for the blood tests.

WHAT DOES THE STUDY ENTAIL?

We routinely take blood from the umbilical cord after the baby is delivered to measure the oxygen level. This is done for all deliveries. For this study, additional small samples of blood will also be retained (about a teaspoonful). We will use this blood to measure the glucose levels and other related substances, which are used for energy. It does not involve taking blood from you or your baby.

WHAT ARE THE DISCOMFORTS, RISKS AND SIDE EFFECTS?

There are no risks to your baby as the blood sample will be taken from the umbilical cord after delivery of the placenta.

WHAT WILL HAPPEN TO THE INFORMATION COLLECTED IN THE STUDY?

All aspects of the study concerning you and your baby will remain confidential. Only the project researchers will have access to the information collected in the study. The information about you will be stored in a locked computer file accessed by password. Your name will not be entered in this file, only a unique record number. Only a limited number of researchers in Dundee will know the names of the participants. A floppy disc containing unique record numbers and names will be kept in a locked filing cabinet.

WHAT ARE MY RIGHTS?

Participation in this study is entirely voluntary and you are free to refuse to take part or to withdraw from the study at any time without having to give a reason and without this affecting your future medical care or your relationship with medical staff looking after you. You are free to discuss the study and seek advice about participation from others.

There will be no payment for participation.

We believe it is safe for you to take part in this study but you are insured through NHS Tayside should something go wrong and compensation is needed.

The Tayside Committee on Medical Research Ethics, which has responsibility for scrutinising all proposals for medical research on humans in Tayside, has examined the proposal and has raised no objections from the point of view of medical ethics. It is a requirement that your records in this research, together with any relevant medical records, be made available for scrutiny by monitors from NHS Tayside and the Regulatory Authorities. But should you not wish this you can refuse and this information will be recorded on your consent form.

A copy of this information sheet and consent form will be given to you to keep. Should you require more information or have any questions, you could contact Dr. Daisy Koh, the main co-ordinator of this study at Ninewells Hospital on 01382 660111 Bleep 4165.

METABOLIC AND HORMONAL RESPONSES TO HYPOGLYCAEMIA IN INFANTS DELIVERED BY CAESAREAN SECTION

(Reference number 033/04)

We invite you to participate in a research project. We believe it to be of potential importance. However, before you decide whether or not you wish to participate, we need to be sure that you understand firstly why we are doing it, and secondly what it would involve if you agreed. We are therefore providing you with the following information. Read it carefully and be sure to ask any questions you have, and, if you want, discuss it with outsiders. We will do our best to explain and to provide any further information you may ask for now or later. You do not have to make an immediate decision.

THE BACKGROUND TO THE STUDY

Every year, there are a few babies who suffer from brain damage at delivery. There are a number of possible causes. We suspect that low blood glucose (sugar) level at birth is one of the possible causes. We would like to study this further in healthy term infants. If this study shows low blood glucose (sugar) level may be a problem then we will seek a solution to this in the future.

We would like to ask mothers who deliver their infants at term to participate in this initial study.

The University of Dundee pays the salaries of the researchers. We will seek further funds from charities to pay for the blood tests.

WHAT DOES THE STUDY ENTAIL?

We routinely take blood from the umbilical cord after the baby is delivered to measure the oxygen level. This is done for all deliveries. For this study, an additional small sample of blood will also be retained (about a teaspoonful). We also require a blood sample from you (about a teaspoonful) just before and after delivery. No further blood samples are required from you or your baby. We will use this blood to measure the glucose levels and other related substances, which are used for energy, as well as hormones which regulate glucose levels. We shall also abstract from your medical notes information about your health during pregnancy, your progress in labour and about your baby at birth

WHAT ARE THE DISCOMFORTS, RISKS AND SIDE EFFECTS?

There are no risks to your baby as the blood sample will be taken from the umbilical cord after delivery of the placenta. Taking a blood sample from you may cause a little discomfort.

WHAT WILL HAPPEN TO THE INFORMATION COLLECTED IN THE STUDY?

All aspects of the study concerning you and your baby will remain confidential. Only the project researchers will have access to the information collected in the study. The information about you will be stored in a locked computer file accessed by password. Your name will not be entered in this file, only a unique record number. Only a limited number of researchers in Dundee will know the names of the participants. A floppy disc containing unique record numbers and names will be kept in a locked filing cabinet.

This is a study to find out what are the normal levels of glucose and other related substances in cord blood at the time of delivery. It is possible we may find babies who have higher or lower levels than other babies. If we think this may affect the future health of your baby, we will discuss this with you. You will be offered an appointment with Professor Robert Hume Consultant Paediatrician at Ninewells to discuss this further. We will not tell your GP that you are taking part in this study but if there is concern about your baby's health we will involve your GP. If your baby's results are similar to other babies in the study we will not routinely tell you the results.

WHAT ARE MY RIGHTS?

Participation in this study is entirely voluntary and you are free to refuse to take part or to withdraw from the study at any time without having to give a reason and without this affecting your future medical care or your relationship with medical staff looking after you.

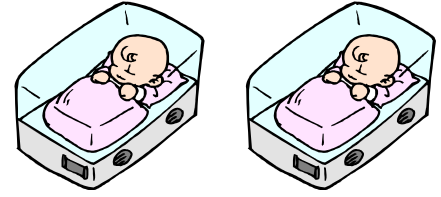
You are free to discuss the study and seek advice about participation from others. There will be no payment for participation.

We believe it is safe for you to take part in this study but you are insured through NHS Tayside should something go wrong and compensation is needed.

The Tayside Committee on Medical Research Ethics, which has responsibility for scrutinising all proposals for medical research on humans in Tayside, has examined the proposal and has raised no objections from the point of view of medical ethics. It is a requirement that your records in this research, together with any relevant medical records, be made available for scrutiny by monitors from NHS Tayside and the Regulatory Authorities. But should you not wish this you can refuse and this information will be recorded on your consent form.

A copy of this information sheet and consent form will be given to you to keep. Should you require more information or have any questions, you could contact Dr. Daisy Koh, the main co-ordinator of this study at Ninewells Hospital on 01382 660111 Bleep 4165.

Metabolic & Hormonal Responses to Hypoglycaemia in Infants delivered by C/S



BACKGROUND INFORMATION

- 1 Mother's surname _____
- 2 Mother's CHI or ID number
- 3 Gestation at delivery completed weeks
- 4 Current smoker ₁ Non-smoker ₂ Ex-smoker ₃
- 5a Booking Weight (Kg) 5b Booking height (m)
- 6a Delivery Weight (Kg) 6b Delivery height (m)
- 7 Date & time last meal ____/____/____
dd mm yyyy 24 hour clock

MATERNAL MEDICAL HISTORY

- 8 ANTEPARTUM HAEMORRHAGE ^g Yes ₁ No ₂
- if yes, episode 1 at gestation weeks
- episode 2 at gestation weeks
- episode 3 at gestation weeks
- > 3 episodes

what was the cause?

Tick if yes

- abruption ₁
- placenta praevia ₂
- vasa praevia ₃
- other ₄ please specify _____
- Not known ₅

9 ASTHMA ^g

- Yes ₁ No ₂ specify treatment _____

10 EPILEPSY ^g

- Yes ₁ No ₂ specify treatment _____

^g Antepartum haemorrhage is vaginal bleeding after 24 weeks
Asthma and epilepsy should be considered present if the women is on current treatment

Yes No
☐₁ ☐₂

11 THYROID DISEASE *concurrent with pregnancy*

Tick all that apply

a if yes,

hyperthyroid	<input type="checkbox"/> ₁
hypothyroid	<input type="checkbox"/> ₁
simple non-toxic goitre	<input type="checkbox"/> ₁
other thyroid enlargement	<input type="checkbox"/> ₁
auto immune thyroiditis	<input type="checkbox"/> ₁
Other, <i>please specify</i>	<input type="checkbox"/> ₁

b Current T4 treatment Yes ☐₁ No ☐₂ **if yes,** T4 mg/day/Last menstrual period
T4 mg/day/delivery

c Family history thyroid disease Yes ☐₁ No ☐₂ **if yes,** Mother ☐₁
Father ☐₁
Siblings ☐₁ out of

d Hyperthyroid treatment Yes ☐₁ No ☐₂ **if yes:**

	Regimen <i>prior</i> to pregnancy	Regimen <i>during</i> Pregnancy
Carbimazole	<input type="checkbox"/> ₁	<input type="checkbox"/> ₁
Surgery	<input type="checkbox"/> ₁	<input type="checkbox"/> ₁
Thiouracil	<input type="checkbox"/> ₁	<input type="checkbox"/> ₁
radioactive iodine	<input type="checkbox"/> ₁	<input type="checkbox"/> ₁

12 DIABETES MELLITUS Yes ☐₁ No ☐₂

a if yes, Insulin dependent diabetes mellitus ☐₁
or, Gestational diabetes: insulin required ☐₂
or Gestational diabetes: non-insulin ☐₃

b Age IDD diagnosed yrs

c IDD insulin at booking units/day

d IDD insulin at delivery units/day

e Gestational diabetes fasting blood glucose mmol/l

f Gestational diabetes 2 hour blood glucose mmol/l
after 75g OGTT

g Gestational diabetes diagnosis made at _____ completed weeks gestation

h Insulin for Gestational diabetes started at _____ completed weeks gestation

i Gestational diabetes max insulin dosage units/day Diet only ☐₁

13 HYPEREMESIS Yes ☐₁ No ☐₂

If yes, tick as many as apply admitted ☐₁

Ketonuria ☐₁

IV fluids given ☐₁

14 ANY OTHER CONDITIONS please specify _____

15 a DID THE MOTHER HAVE GROUP B STREPTOCOCCUS Yes ☐₁ No ☐₂

If yes

b Group B identified: Previous pregnancy ☐₁ Current pregnancy ☐₂

e At what gestation at current pregnancy was Group B identified weeks

c If yes was treatment tick as appropriate antenatal ☐₁ intrapartum ☐₂

d Antibiotic please specify _____

MATERNAL MEDICATIONS

16 a Ritodrine Yes ☐₁ No ☐₂

e total ritodrine dosage (mg) _____

g Date & time final dose / /
dd mm yyyy 24 hour clock

17 a Tractocile Yes ☐₁ No ☐₂

b initial / /
dd mm yyyy 24 hour clock

d total days^g treatment

e total tractocile dosage (mg) _____

c final^g / /
dd mm yyyy 24 hour clock

18 a First Steroid Course Yes ☐₁ No ☐₂

b initial / /
dd mm yyyy 24 hour clock

d total steroid dosage (mg) _____

if yes, Name of steroid _____

c final^g / /
dd mm yyyy 24 hour clock

^g Final date is the date is the final date of treatment or the date of delivery whichever is first

- 19 a Second Steroid Course** Yes ☐₁ No ☐₂ if yes, Name of steroid _____
- b** initial ____/____/____ ☐☐☐☐ ☐☐☐☐
dd mm yyyy 24 hour clock
- c** final^g ____/____/____ ☐☐☐☐ ☐☐☐☐
dd mm yyyy 24 hour clock
- d** total steroid dosage (mg) _____

MATERNAL HYPERTENSION during pregnancy^g

- 20** Is the mother hypertensive? Yes ☐₁ No ☐₂ →

Go to Q27
- a** Chronic Hypertension ☐₁
- b** Gestational Hypertension (>140/90 after 20 weeks) ☐₂
- c** Preeclampsia (PET) (HPT + proteinuria) ☐₃
- d** PET superimposed on chronic HPT ☐₄

- 21** 24-hour urine collection for proteinuria Yes ☐₁ No ☐₂
- if yes, urine protein ☐•☐☐ g/L ☐•☐☐ g/d
- 22** BP prior to delivery: **a** Systolic BP ☐☐☐ mmHg **b** Diastolic BP ☐☐☐ mmHg
- 23** PET bloods taken prior to delivery Yes ☐₁ No ☐₂
- If yes,
- a** Gestation blood taken ☐☐ weeks
- b** Urea ☐•☐ mmol/l **c** Creatinine ☐☐☐ μmol/l
- d** Urate ☐•☐☐ mmol/l **e** ALT ☐☐☐ U/l
- f** Bilirubin ☐☐☐ μmol/l **g** Albumin ☐☐☐ g/l
- h** Platelet ☐☐☐ 10⁹/l

ANTI HYPERTENSIVE DRUGS during pregnancy ONLY

NB final date is date finish treatment OR delivery WHICH EVER IS FIRST

- 24** **a** First drug please specify _____
- b** start ____/____/____ ☐☐☐☐ **c** final ____/____/____ ☐☐☐☐
dd mm yyyy dd mm yyyy
- d** Dose per day _____
- 25** **a** Second drug please specify _____

b start ____/____/____
dd mm yyyy

c final ____/____/____
dd mm yyyy

d Dose per day _____

26 a Third drug *please specify* _____

b start ____/____/____
dd mm yyyy

c final ____/____/____
dd mm yyyy

d Dose per day _____

MATERNAL ANTICONVULSANT DRUG *during pregnancy ONLY*

NB final date is date finish treatment OR delivery WHICH EVER IS FIRST

27 a Drug 1 *please specify* _____

b start ____/____/____
dd mm yyyy

c final ____/____/____
dd mm yyyy

28 a Drug 2 *please specify* _____

b start ____/____/____
dd mm yyyy

c final ____/____/____
dd mm yyyy

29 a Drug 3 *please specify* _____

b start ____/____/____
dd mm yyyy

c final ____/____/____
dd mm yyyy

ANY (ORAL OR IV) MATERNAL STEROIDS (eg for asthma) other than dexamet etc. for prevention of RDS.⁹

Course 1

30 a Name _____

B start⁹ ____/____/____
dd mm yyyy

c finish⁹ ____/____/____
dd mm yyyy

D total daily dosage _____ mg

e total days treatment

f maternal weight start treatment • kg

Course 2

31 a Name _____

B start⁹ ____/____/____
dd mm yyyy

c finish⁹ ____/____/____
dd mm yyyy

D total daily dosage _____ mg

e total days treatment

f maternal weight start treatment • kg

Course 3

- 32 a** Name _____
- B** start^g ____/____/____
dd mm yyyy
- c** finish^g ____/____/____
dd mm yyyy
- D** total daily dosage _____ mg
- E** total days treatment
- f** maternal weight start treatment • kg

TREATED MATERNAL GU INFECTIONS (*In the week prior to delivery*)

- 33**
- A** Urinary tract infection ☐₁ ☐₂ Yes No **if yes,**
- b** Treatment (*specify*) _____ **c** Dosage _____
- d** Organism (*specify*) _____ **e** Date ____/____/____
- 34**
- A** Vaginal discharge ☐₁ ☐₂ Yes No **if yes,**
- b** Treatment (*specify*) _____ **c** Dosage _____
- d** Organism (*specify*) _____ **e** Date ____/____/____

DELIVERY AND BIRTH DETAILS

Elective caesarean section

- 35** Reasons for C/S
- | | |
|----------------------------|---------------------------------------|
| Previous caesarean section | <input type="checkbox"/> ₁ |
| Breech presentation | <input type="checkbox"/> ₂ |
| Maternal request | <input type="checkbox"/> ₃ |
| Placenta praevia | <input type="checkbox"/> ₄ |
| Other | <input type="checkbox"/> ₅ |
- Please specify _____

- 36** Drugs received during labour **tick as many as apply**
- | | | | |
|------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|
| a None received | <input type="checkbox"/> ₁ | d Epidural / spinal | <input type="checkbox"/> ₁ |
| b Entonox | <input type="checkbox"/> ₁ | e Opiate | <input type="checkbox"/> ₁ |
| c General anaesthesia | <input type="checkbox"/> ₁ | f Other, <i>please specify</i> | <input type="checkbox"/> ₁ |
-

- 37 a** Type of fluid during caesarean section prior to delivery _____
- b** Total volume _____

- 38** Placental weight gm

DELIVERY OUTCOMES

39a Date of birth ____/____/____

40 Apgar score 1 minute

42 Resuscitation required Yes ☐ ₁ No ☐ ₂

if yes, Narcan and/or facial O₂ ☐ 1
IPPV by mask ☐ 2

39b Time of birth 24 hour clock

41 Apgar score 5 minutes

IPPV by tube	<input type="text"/>	3
IPPV and drugs	<input type="text"/>	4

INFANT CHARACTERISTICS

43 Infant's Surname _____

44 Infant's DoB / /
dd mm yyyy

45 Infant's sex Male ☐ ₁ Female ☐ ₂

46 Infant's birth weight gm

47 Birth length cm

48 Birth OFC . cm

MATERNAL BIOCHEMISTRY

49a Glucose (1st) mmol/l

24 hour clock

49b Lactate (1st)

mmol/l

24 hour clock

50a Glucose (2nd) mmol/l

24 hour clock

50b Lactate (2nd) mmol/l

24 hour clock

INFANT BIOCHEMISTRY

Time blood taken
24 hour clock

Umbilical artery

51a pH

b pCO₂ kPa

c pO₂ kPa

d cHCO_3^- mmol/l

e BE  mmol/l

53 Glucose mmol/l

Umbilical vein

52a pH

b pCO₂ kPa

c pO₂ kPa

d CHCO_3 mmol/l

e BE  mmol/l

54 Lactate mmol/l

55 Cortisol	<input type="text"/> <input type="text"/> <input type="text"/> nmol/l	56 GH	<input type="text"/> <input type="text"/> <input type="text"/> ng/ml
57 Insulin	<input type="text"/> <input type="text"/> <input type="text"/> mU/l	58 Glucagon	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> ng/l
59 3-hydroxybutyrate	<input type="text"/> <input type="text"/> <input type="text"/> mmol/l	60 Free fatty acids	<input type="text"/> <input type="text"/> <input type="text"/> umol/l
61 Catecholamine	pg/ml		
a. DHPG	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	b. DHPG sulfate	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
c. NE	<input type="text"/> <input type="text"/> <input type="text"/>	d. NE sulfate	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
e. DOPA	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	f. DOPA sulfate	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
g. EPI	<input type="text"/> <input type="text"/> <input type="text"/>	h. EPI sulfate	<input type="text"/> <input type="text"/> <input type="text"/>
i. Dopamine	<input type="text"/> <input type="text"/> <input type="text"/>	j. Dopamine sulfate	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
k. DOPAC	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	l. DOPAC sulfate	<input type="text"/> <input type="text"/> <input type="text"/>

a ☐ ₁ Yes ☐ ₂ No **if yes, Name of steroid**

b initial / /
dd mm yyyy

c final⁹ / /
dd mm yyyy

d total steroid dosage (mg)

f Date & time final dose _____/_____/_____
dd mm yyyy 24 hour clock

a

Yes

No

if yes, Name of steroid

b initial / / /
dd mm yyyy

c final^g / / /
dd mm yyyy

d total steroid dosage (mg)

f Date & time final dose _____/_____/_____
dd mm yyyy 24 hour clock

a ☐ ₁ Yes ☐ ₂ No **if yes, Name of steroid**

b initial / / **c** final⁹ / / **d** total steroid dosage (mg)

f Date & time final dose _____/_____/_____
dd mm yyyy
24 hour clock

CONSENT FORM

NB. This form must be completed and signed by the research subject in the presence of someone with knowledge of the research designated by the Principal Investigator. This may be a doctor, nurse, clinical research assistant or other member of the research team who must countersign the form as witness to the subject signature.

Please tick (✓) appropriate box

Have you read and understood the Subject Information Sheet? Yes ☐ No ☐

Have you been given an opportunity to ask questions and further discuss this study?
Yes ☐ No ☐

Have you received satisfactory answers to all of your questions? Yes ☐ No ☐

Have you now received enough information about this study? Yes ☐ No ☐

Who have you spoken to? Dr/Mr/Mrs/Miss

Do you understand that your participation is entirely voluntary? Yes ☐ No ☐

Do you understand that you are free to withdraw from this study:

At any time? Yes ☐ No ☐

Without having to give a reason for withdrawing? Yes ☐ No ☐

Without this affecting your present or future medical care? Yes ☐ No ☐

Do you agree that your records in this research and supporting medical records be made available for inspection by monitors from:

University of Dundee Yes ☐ No ☐

NHS Tayside monitors? Yes ☐ No ☐

Regulatory authorities? Yes ☐ No ☐

Do you agree to take part in this study? Yes ☐ No ☐

Do you agree to any blood used in this study being retained for use in future research? Yes ☐ No ☐ Not applicable ☐

Subject's signature Date

Subject's name in block capital letters

Telephone contact (Patient) (Home) (Work)

Signature witnessed by Date.....

Witness name in block capital letters